EPIDEMIOLOGICAL STUDIES OF VEROCYTOTOXIN-PRODUCING *ESCHERICHIA COLI* INFECTIONS IN ANIMALS IN SCOTLAND

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ABSTRACT

This thesis is a summation of studies carried out between 1991 and 2004 and attempts to place the work in context with other knowledge to establish the role of animals as a source of human infection with verocytotoxin-producing *Escherichia coli* (VTEC).

In a preliminary prevalence study, VTEC O157 was first isolated from cattle in Scotland in 1992. In this study, using basic techniques to examine faeces samples routinely submitted to the Scottish Agricultural College (SAC) Veterinary Centres, 0.25% samples from cattle were positive for VTEC O157. The organism was more commonly isolated from calves less than two months of age.

A very large prevalence study was commissioned following the Central Scotland outbreak. Using what has now become the national reference method (immunomagnetic separation following enrichment of 1g faeces in buffered peptone water with no antibiotics), prevalence levels were established with 95% confidence limits as follows. 7.9% (6.5%-9.6%) animals sampled (12-30 months of age) were shedding VTEC O157. 22.8% (19.6%-26.3%) of farms had at least one animal shedding in the group sampled. There was a significant drop in the proportion of farms where shedding was detected between the three years of the study 1998, 1999 & 2000. When farms were repeatedly visited twelve times, the organism was detected on 87.5% farms. Because of the lack of sensitivity of the test and the uneven distribution of the organism in faeces, these are underestimates of the true prevalence.

In a cohort study in beef finishing cattle and a longitudinal study in beef cows risk factors for shedding VTEC O157 were determined from questionnaires followed by univariate and multivariate analysis. Increased levels of shedding were associated with animals being housed rather than grazing. Farms with animals at pasture have lower prevalences if the water is from a natural source. The presence of wild geese was also seen as a risk factor. Farms that spread slurry on grazing land were more likely to have shedding animals. Larger farms were more likely to be positive. There were no significant regional differences in shedding within Scotland.

A pilot prevalence study in sheep determined a Group Level Prevalence of 8% with 95% confidence of 2% to 19%) and an Animal Level Prevalence of 1%). Enumeration of VTEC O157 organisms gave counts ranging from $<5x10^2g^{-1}$ to $>10^4g^{-1}$. A similar study in deer in Scotland suggested that the prevalence was low.

Ninety-one farm investigations were carried out associated with 57 incidents of human *E. coli* O157 infection, when animals were suspected as a possible source. In eighteen instances an indistinguishable isolate was obtained from an animal to a human case. The most likely modes of transmission were postulated in each incident. The routes included improperly pasteurised milk, raw milk, direct contact with cattle, contact with a contaminated environment, contaminated camp sites by cattle or sheep, untreated private water supplies contaminated by sheep, cattle or deer and contact with a cat on a farm.

Finally it is postulated that the regional variation in the rate of infection per unit population in Scotland and the difference between Scotland and England relate to the relative cattle and human populations in the areas being considered.

KEY WORDS

E. coli, O157, VTEC, cattle, sheep, deer

STATEMENT OF ORIGINALITY

The compilation of this thesis and the work contained therein has been conducted by the author and has not been submitted in candidature of another qualification. Where relevant, acknowledgement has been made of collaboration with colleagues. The data collection has been conducted in accordance with the European Union Guidelines for Good Clinical Practice (GCPV) (Anon, 1994)

Barti A Synge

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The author participated in three International Symposia on Shiga Toxin (verocytotoxin) – producing *Escherichia coli* Infections, in Bergamo, Kyoto and Edinburgh and also the European Concerted Action (*CT98-3935 Verocytotoxigenic E.*

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Chapter 1: INTRODUCTION & METHODS

Verocytotoxin-producing *Escherichia coli* (VTEC) O157 was responsible for twenty deaths in a single outbreak in central Scotland in 1996/7. Fortunately there have been no large outbreaks in recent years but severe illness and deaths still occur. Approximately two hundred cases are reported in Scotland every year, four cases per hundred thousand of human population - a rate three times higher than that in England and Wales. From the mid nineteen-eighties livestock were considered to be a potential source of infection for man. Studies of the epidemiology of the organism in livestock are still considered to be helpful in the fight to control human infection.

This thesis is designed to present in one place the work carried out by the author on VTEC O157 in animals in Scotland from 1990 to 2005. The contributions of many colleagues have already been acknowledged. Each chapter outlines a different phase of the work. Where the material has been published this is clearly stated at the end of the chapter. The work presented is where Synge has been the primary author. Other papers where colleagues have been primary authors are included in the list of publications in the appendix.

Chapter 2 is a literature review of VTEC O157 from a veterinary perspective published in 2000. The following chapters cite further papers and a bibliography is presented all together at the end of the thesis. Chapter 3 describes an early prevalence study in a variety of animals. Prevalence studies in cattle, sheep and deer are described in chapters 5, 6 and 7 respectively. Risk factors that influence the shedding of VTEC O157 are analysed in the longitudinal study in beef cows and the prevalence study in finishing beef cattle in chapters 4 and 5. Chapter 8 reviews 91 farm

investigations carried out between 1993 and 2003, associated with 57 incidents where animals were a possible source of infection for humans. Finally in chapter 9 there is a discussion which attempts to put the work in perspective with recent publications and ongoing work. An analysis attempts to explain why Scotland has three times the level of human infection compared to England and Wales.

Methods

While the methods used for each study are described in each chapter a brief overview is given here. The methods used for bacterial isolation and typing developed rapidly in the early period of study (Synge, 1998).

(a) Direct Culture

In the initial study described in Chapter 3 faeces samples and rectal swabs were screened using the method available at the time, namely plating on sorbitol MacConkey agar and selecting non-sorbitol fermenting colonies for latex agglutination. Positively agglutinating colonies were tested for verocytotoxic effect on vero cells by the method of Chapman and Swift (1984).

(b) Pilot Study on the use of antibiotics in the enrichment medium preceding Immuno-magnetic separation.

At the outset of the project there was some conflicting evidence regarding the most sensitive isolation method for VTEC O157. Immunomagnetic separation had been shown to be more sensitive than direct culture (Chapman, *et al.*, 1994), but Hopkins, working at SAC Thurso, had observed higher isolation rates when the antibiotics were left out of the enrichment broth. Samples were tested in parallel with the inclusion of cefixime, cefsulodin & vancomycin or no antibiotics in the phosphate buffered peptone water. The results from 721 samples taken from 46 farms showed

conclusively that significantly more isolates were achieved without antibiotics (Foster, *et al.*, 2003) and this method was adopted for the studies described in Chapters 4 to 8 and has been adopted as the national reference method for the isolation of VTEC O157 from animal faeces. The method is described in Chapter 5. In all the studies using this method, testing a one gram faeces sample has been standard. This is reviewed in the light of new knowledge in Chapter 9.

(c) Typing

In the initial prevalence study (Chapter 3)verocytotoxin production was detected with verocells by the method of Chapman and Swift (1984). Thereafter genes encoding the production of verocytotoxins (VT1 & VT2) and enterocyte attachment and effacement (eae) were detected by multiplex polymerase chain reaction (Louie, *et al.*, 1994, Pollard, *et al.*, 1990). Isolates were phage typed using standard methods (Khakria, *et al.*, 1990). In addition to determine linkage between human incidents and animal isolates plasmid profiling was initially carried out at the Scottish Salmonella Reference Laboratory. The molecular weight of each plasmid found was expressed in kilodaltons and each recognisable fingerprint was expressed by a letter a, b, c etc. as shown in Table 2.1. Pulsed-field gel-electrophoresis has been adopted however as the standard method to differenciate or associate isolates in Scotland (Allison, *et al.*, 2000).

Table 2.1 Use of typing methods to distinguish between human isolate and five isolates from cattle on three adjacent farms.

Isolate	Phage type	Vercytotoxins	Plasmid Profile	Pule Field
Child	2	VT1-ve VT2+ve	90e : 70 : 10	Standard pattern
Farm A i)	2	VT1-ve VT2+ve	90e : 70 : 10	Indistiguishable
ii)	2	VT1-ve VT2+ve	85l	Distinguisable
Farm B	8	VT1+ve VT2+ve	85h	Distinguisable
Farm C i)	8	VT1+ve VT2+ve	85h	Distinguisable
ii)	2	VT1-ve VT2+ve	90e : 70 : 10	Indistiguishable VT1+ve VT2+ve

Chapter 2: VETERINARY SIGNIFICANCE OF VEROCYTOTOXIN-PRODUCING *ESCHERICHIA COLI* O157 (A Literature review)

Summary

Verocytotoxin-producing *Escherichia coli* O157 is a serious pathogen in man that is carried by ruminants and has been isolated from some other animal species. Except in the very young of certain species and in greyhounds, the organism is not associated with disease in animals. Humans may be infected by ingestion of the organism through direct animal contact, from contaminated food or water or from the environment. Great efforts have been made to improve hygienic food production and handling, to protect water supplies and to give adequate advice to people handling animals. It is also essential to try to reduce the numbers of organisms shed by animals and, to do this, a clear understanding of the ecology of the organism is required.

Introduction

Verocytotoxin-producing *E. coli* (VTEC) O157:H7, or its immotile form (H-), is a serious pathogen in man (Chart, 2000). Apart from a condition described in greyhounds and in some very young animals, VTEC O157 is not a significant animal pathogen. However, *E. coli* O157 has enormous veterinary public health significance because the agent is a potential zoonosis, and may be transmitted to man through foods, water or by direct contact. Since ground beef was first implicated as a source of infection, in the form of improperly cooked hamburgers, interest has focussed upon animal reservoirs. It is considered that by studying the epidemiology in animals, it may be possible to devise methods of control to reduce the possibility for direct

infection or contamination of meat, dairy products, vegetables and water supplies that can all be contaminated by animal faeces. Sharp *et al.* (1995), when giving an epidemiological overview of *E. coli* O157 in Scotland 1984-94, commented on the importance of interdisciplinary collaboration, in particular the close working relationship among microbiologists, public health physicians, veterinarians and epidemiologists. Great progress has been made and especially in countries where such teamwork exists. Recently, thanks to the generosity of the Wellcome Trust, an exciting new International Partnership in Veterinary Epidemiology has been formed that adds mathematical modellers, evolutionary and molecular biologists, and ecologists to a team trying to unravel the mysteries of Enterobacteriaceae. The interest in this subject over the last two decades has been so immense that this review cannot be comprehensive but it seeks to cover areas of veterinary importance with which the author is most familiar. Non-O157 VTEC are not covered here but have been reviewed elsewhere (Bettelheim, 2000).

VTEC O157 in ruminants

Soon after VTEC O157 was first associated with haemolytic uraemic syndrome (HUS) in man (Riley, *et al.*, 1983), cattle were identified as carriers in the USA (Martin, *et al.*, 1986) and Canada (Borczyk, *et al.*, 1987). Smith *et al.* (1987) also pointed out the relationship of this organism with haemorrhagic colitis in man in England and Wales, while Chapman *et al.* (1989) made the first English isolations and Synge & Hopkins (1992) detected the organism in diarrhoeic calves in Scotland. However Orskov *et al.* (1987), who established the somatic antigen O157 in 1972, detected VTEC O157 in one to three-week-old calves with colibacillosis on four

farms in Argentina, sampled in 1977. These authors did not find this strain in any other animal isolates at this time.

Montenegro (1990) isolated VTEC O157 from healthy cattle in Germany and the organism has been found in cattle in many countries, for example Australia (Cobbold and Desmarchelier, 2000), Japan (Itoh, *et al.*, 1999), Switzerland (Stephan, *et al.*, 2000) and Norway (Vold, *et al.*, 1998).

Following the independent identification in Australia, Great Britain and the USA that sheep meat could also be a source of VTEC O157 for man, Chapman & Siddons (1996) isolated the organism from sheep rectal swabs taken immediately post slaughter. The organism has been isolated from goats in follow-up studies of human infection (Smith, *et al.*, 1998). Incidents involving infection acquired from sheep in Scotland are discussed in Chapter 8 and a pilot prevalence study in sheep is described in Chapter 6.

Wild deer sharing rangeland with cattle were found to be carriers in the USA (Rice, *et al.*, 1995) and, in the UK, Chapman & Ackroyd (1997) found the organism in farmed deer. This particular farm had not kept other types of livestock for the last twenty years. The significance of deer as carriers is discussed in Chapter 8 and a pilot prevalence study in Chapter 7.

VTEC O157 in other animal species

VTEC O157 has been isolated from pigs (Chapman, 2000) during on-farm investigations. Surveys of pigs however do not suggest it as a major reservoir of

infection. *E. coli* O157 isolates from pigs are frequently not verocytotoxin producers (Chapman, *et al.*, 1997) but they can be enterotoxigenic possessing the F4 (K88) adhesin (Wray, *et al.*, 1993). VTEC that cause oedema disease in pigs carry the gene encoding a specific verocytotoxin VT2e and this is not associated with *E. coli* O157. However, Heuvelink *et al.* (1999) have isolated potentially pathogenic VTEC O157 from Dutch slaughter pigs. It is perhaps only a matter of time before more pigs are found to carry this organism.

VTEC O157 has been isolated from other domestic animals, for example, horses (Chalmers, *et al.*, 1997, Trevena, *et al.*, 1996) and dogs (Trevena, *et al.*, 1996), especially greyhounds (Cowan, *et al.*, 1997). The organism was isolated from zoo animals including primates (Bauwens, *et al.*, 2000). Natural infection of poultry has not been reported, but the organism was isolated from domestic geese by the Scottish Agricultural College in a human follow-up study (Smith, *et al.*, 1998), from turkeys in Holland (Heuvelink, *et al.*, 1999) and from seagulls (Wallace, *et al.*, 1997). House flies (Hancock, *et al.*, 1998, Iwasa, *et al.*, 1999) and fruit flies (Janisiewicz, *et al.*, 1999) have been shown to carry the organism.

Pathogenicity of *E. coli* O157 and animals as models for human infection

E. coli O157 is the agent most commonly associated with HUS in man, especially children. Virulence is attributed to the production of verocytotoxins and its ability to intimately adhere to the enterocyte cell membrane of the host by an attaching and effacing mechanism. The genes encoding the verocytotoxins are described as VT1 and VT2, while the *eae* gene encodes the mechanisms for attachment and effacement.

E. coli strains that contain either VT gene plus *eae* are known as enterohaemorrhagic *E. coli* (EHEC) and those that contain *eae* but are not VTEC are termed enteropathogenic *E. coli* (EPEC). Studies in Scotland suggest that the vast majority of VTEC O157 also have *eae* and so are EHEC. Synge (2000) looked at VTEC in general in animals.

There is little field evidence that *E. coli* O157 causes clinical disease in animals. One study (Synge and Hopkins, 1994) identified scouring calves shedding the organism but other pathogens were present and Richards *et al.* (1998) found the organism most frequently in animals without enteric disease. Occasional field cases of enteritis have been associated with other VTEC, for example serotype O26 was isolated from two-week-old calves with dysentery (Anon, 2000) and attachment and effacement was shown with the same serotype in an eight-month-old heifer (Pearson, *et al.*, 1999). Greyhounds are susceptible to a naturally occurring disease, cutaneous and renal glomerular vasculopathy (CRGV), where thrombotic vascular lesions occur in the skin and kidneys. The glomerular lesions and clinical pathology of CRGV closely resemble HUS in man (Cowan, *et al.*, 1997).

Experimental studies have attempted to determine if the organism colonises and is pathogenic for cattle. Dean-Nystrom *et al.* (1997) inoculated calves less than thirty-six hours old with VTEC O157 and produced diarrhoea associated with attaching and effacing lesions in both the small and large intestine. In other studies where older calves were infected artificially (Cray and Moon, 1995, Woodward, *et al.*, 1999), attaching and effacing lesions were produced but without clinical effects. Wray *et al.* (2000) described experimental infection in naturally infected cattle and found no

evidence of adherence to the mucosa but in three out of six animals there was an increase in IgG after challenge. Neonatal calves are not useful models for the study of the disease in man because of the difficulties involved in reproducing the condition. There is no evidence of pathogenicity in other ruminants.

The infant rabbit (five to ten-days-old) was the first model of *E. coli* O157 induced diarrhoea to be developed, and very extensive studies have been carried out, including the use of vaccines to protect against the uptake of verocytotoxins. Mice have also been used to study the effects of parenteral injection of verocytotoxin. A gnotobiotic pig model developed by Francis *et al.* (1986), and Tzipori *et al.* (1995), demonstrated that intimate bacterial attachment was not a prerequisite for VT2 transfer from the gut lumen to the circulation. One-day-old chicks inoculated in the crop with VTEC O157 developed disease, and attachment and effacement was detected in the caecal mucosa. Under experimental conditions hens have become chronic shedders (Schoeni and Doyle, 1994). An overview of animal models is given by Moxley & Francis (1998).

The Prevalence of VTEC O157 in Livestock

Studies of prevalence are important in helping to ascertain the risk of human infection but because of different methodologies great care is required when comparing figures from different studies. As *E. coli* O157 is usually present as a minority organism and in low numbers, the detection method is of great importance. In early studies direct plating on to sorbitol MacConkey agar was used. Synge & Hopkins (1996) found only 0.25% positive out of more than 5000 bovine samples submitted to veterinary investigation laboratories throughout Scotland, whilst Chapman *et al.* (1993), by a similar method, found 4% cattle to be carriers at an abattoir in Sheffield, England. A

study of diagnostic samples in England and Wales, similar to the Scottish study but using immuno-magnetic separation (IMS), found 0.83% bovines positive and a later study in the Sheffield abattoir, again using IMS, identified 15.7% of cattle as carriers. In both studies using IMS the prevalence increased by fourfold and is likely to be the result of using a more sensitive technique rather than an increase of prevalence with time (Chapman, *et al.*, 1994). A major prevalence study in Scottish beef cattle, aged between 12 and 30 months, has just been finished and the preliminary analysis suggests that 24% of herds had at least one animal shedding and 8.6% of animals were positive (Synge and Paiba, 2000). The studies in England and Wales described in the same publication show up to 50% herds may be shedding the organism but this was in a wider sampling frame.

Studies in the USA have shown similar trends with improved techniques. Hancock *et al.* (1994), using direct plating to sorbitol MacConkey, isolated *E. coli* O157 from 8% of dairy herds and 16% of pastured beef herds. A later study by the same group, using broth enrichment but not IMS, showed nine out of fourteen herds to be positive (Hancock, *et al.*, 1997). In a Dutch study 10 dairy farms were visited and all the cattle on the farm were sampled and tested by IMS. Seven out of ten farms were positive for VTEC O157, with the proportion of positive animals varying from 0.8 - 22.4% (Heuvelink, *et al.*, 1998). Thus it must be assumed that in many countries the majority, but not necessarily all, farms have some animals shedding the organism. It remains unclear if there are countries where the organism is not present.

The prevalence of VTEC O157 in animal meats has also been examined. Heuvelink *et al.* (1999), in the Netherlands, found VTEC O157 in 1% of raw minced beef and 1%

of raw pork products, while raw products from poultry, lamb and wild animals were all negative. In the UK 1.4% of 5000 beef and lamb products were contaminated (Chapman, *et al.*, 2000).

Factors affecting the prevalence of *E. coli* O157 shedding

Age has an effect. Synge & Hopkins (1996) found more calves less than two-months-old shedding than older calves, which in turn had less shedding than adults. Hancock *et al.* (1997) found a higher prevalence (1.8%) in weaned heifers than in unweaned calves (0.9%) and adults (0.4%). Seasonal effects have also been described and in Canada and the USA higher prevalences are reported in the summer months (Clarke, *et al.*, 1994, Hancock, *et al.*, 1997). In a longitudinal study of a single dairy herd in England, Mechie *et al.* (1997) found a peak of shedding in the first month of lactation with a peak between May and July and a smaller peak in November. Synge (1999) found a similar pattern in a beef suckler herd in Orkney, Scotland. In a large study of 953 groups of finishing beef cattle and a longitudinal study of 32 beef suckler herds, the preliminary results suggest shedding is higher in the spring and autumn than in the winter or summer (Synge, *et al.*, 2000). These studies are attempting to elucidate interactions with housing, feeding, calving and weaning etc. that might influence shedding of the organism. However, to date the effects of these interactions are unclear.

In the study of a dairy herd by Mechie *et al.* (1997), *E. coli* O157 persisted in the herd for at least fifteen months, even though there was no detectable shedding of the organism for five months during the winter. Only one cow out of 73 shed the organism more than two weeks consecutively. Synge (2000) found similar results in

Orkney, but in a separate group of intensively managed dairy calves the organism was consistently isolated for seven months. In a Canadian study of eight dairy herds, Rahn *et al.* (1997) concluded that shedding by dairy cattle was transient. In an experimental study, inoculated calves shed the organism intermittently up to 58 days and cows up to 44 days, but on the majority of sampling days the organism was not detected (Wray, *et al.*, 2000). Brown *et al.* (1997) showed that, following inoculation, *E. coli* O157 persists in the rumen, reticulum, omasum and parts of the large colon during the period of faecal shedding. A definitive site of mucosal colonisation was not found. It has been postulated that the persistence of *E. coli* O157 may be increased by its ability to survive within protozoa in the soil and possibly the rumen (Barker, *et al.*, 1999).

Nutritional effects on the shedding of VTEC O157

It has been suggested that the diet, abrupt changes in diet, or fasting may influence the shedding of *E. coli* O157 but many of the results are contradictory. Kudva *et al.* (1997) investigated these hypotheses with experimentally inoculated sheep as a ruminant model. Two diets were investigated; a concentrate that consisted of a mixture of corn and pelleted alfalfa, high in protein, digestible energy and low in fibre; and a grass hay diet, low in protein and digestible energy but high in fibre. Hay fed sheep shed the organism for twice as long and in higher numbers than sheep that had been fed the concentrate. The number of culture positive animals increased when the diet was abruptly changed from concentrate to hay and decreased with the opposite change. Twenty-four hour fasting did not influence *E. coli* O157 shedding. In cattle, Diez-Gonzalez *et al.* (1998) showed that animals, which were fed mostly grain, had a lower colonic pH and more acid resistant *E. coli* than cattle that were fed

only hay. A brief period of hay feeding decreased the acid resistant count substantially. *E. coli* O157 tend to be more acid resistant than many other *E. coli*. However, Hovde *et al.* (1999) suggested that hay fed cattle shed *E. coli* O157 for longer than grain fed animals. Russell *et al.* (2000) concluded that feeding hay for a period of up to seven days decreases the number of cattle shedding *E. coli* O157.

The fate of VTEC O157 in faeces and the environment

Knowledge of the ability of the organism to survive in faeces, on pasture and associated water systems has implications for its spread to crops, which may be a source of infection for man or indeed for animals. Maule (2000) found that the organism survived longest in soil cores containing rooted grass where levels of 10^8 per gram declined to 10^6 per gram after 130 days. The organism was detectable in cattle faeces at high levels for more than fifty days. The organism survived much less readily in slurry or river water. The decline in *E. coli* numbers increases with temperature. Himathongkham *et al.* (1999) showed more rapid destruction at 37°C than at 20°C or 4°C. In manure, reduction times varied between six days and three weeks, while in slurry the times ranged from two days to five weeks. Beuchat (1999) demonstrated survival of the organism on lettuce contaminated by cattle faeces for at least 15 days. Fenlon *et al.* (2000) related the fall off in *E. coli* numbers in soil to penetration to deeper soil layers and to run off into drains following rain. Experimental studies showed that *E. coli* O157 on grass, which was subsequently ensiled in conditions allowing aerobic spoilage, could multiply to numbers exceeding 10^6 per gram in the silage (Fenlon *et al* 2000).

Sources of VTEC O157 for livestock

Cattle can undoubtedly pass *E. coli* O157 from one to another. This is likely to happen in both housed and grazing animals. Recent studies by Synge *et al.* (2000) found more housed animals shedding the organism. Buying in replacements rather than home rearing was also a factor that increased the likelihood of a farm being positive. The possibility of infection from poorly made silage has also been demonstrated (Fenlon, *et al.*, 2000). In the USA, replication of *E. coli* O157 in wet grains and silage/corn mixes has been observed (Lynn, *et al.*, 1998). Cattle or sheep may also acquire infection from wild animals e.g. deer grazing the same pasture (Rice, *et al.*, 1995). Wild birds, especially seagulls and other scavengers, may spread infection from farm to farm, from human sewage or refuse tips to pasture (Wallace, *et al.*, 1997).

Routes of infection for man

Major VTEC O157 outbreaks in man are most usually associated with food. For example, in the USA (Griffin, 1998), food was the most common source- 67%, followed by person to person contact, usually in child care centres- 22%, swimming water- 8% and drinking water- 2%. O'Brien (2000) reviewed the sources of outbreaks in the United Kingdom. These included hamburgers, yoghurt, unpasteurised milk, milk sold as pasteurised, animal contact, various meat products in the large central Scotland outbreaks, cheese made from unpasteurised milk, contaminated mud at a pop festival, a private water supply and a contaminated beach. In the case of outbreaks associated with meat products, it is rarely possible to trace the contamination back to farm level but with the other types of food such as milk or where direct contact has occurred, trace backs have allowed an animal source to be

identified. Synge & Hopkins (1996) isolated indistinguishable VTEC O157 from cattle and humans in ten out of nineteen incidents investigated. Pulsed-field gel electrophoresis was proved to be an enormous benefit in identifying the source.

In Scotland where there have been consistently higher rates of human infection than almost any country, the majority of cases are sporadic rather than related to outbreaks (Reilly, *et al.*, 2000), although Scotland has also suffered some major outbreaks. A case control study in Scotland showed contact with animal faeces, likely contact with animal faeces and animal contact to be highly associated risk factors. In all cases this contact was with animals other than pets (Reilly, *et al.*, 2000). Direct contact with animals is a well-established risk factor; for example with calves (Renwick, *et al.*, 1993, Synge, *et al.*, 1993) or lambing ewes (Allison, *et al.*, 1997).

Outbreaks have also been associated with contaminated fruit or vegetable products; for example unpasteurised apple juice (Cody, *et al.*, 1999). Contaminated water has lead to several outbreaks (Chalmers, *et al.*, 2000). A waterborne outbreak, due to the contamination by sheep of a private water supply from a hillside in a remote area of the Scottish Highlands (Synge, 2000), highlights the ubiquitous nature of the organism.

The prevention of VTEC O157 infection from animals

There is always a potential risk when people have contact with animal faeces. Persons working with animals must be made aware of the risks to themselves and their families, and adequate protective clothing which is not taken home, and hand washing facilities must be provided. The risk appears to be higher on open farms visited by members of the public who are not familiar with animals. The Health and

Safety Executive in the UK have issued advice to farmers and schoolteachers (HSE, 2000).

Much attention has focussed upon the need to produce meat that has a low level of contamination. It is generally considered that there must be farm to fork strategies to reduce the risk of infection, including control strategies at farm level together with hygienic food handling systems and adequate cooking. Gannon (1999) discussed the control of VTEC O157 in cattle at slaughter and advocated the use of a quality control system such as hazard analysis and critical control points (HACCP). However, VTEC O157 presents a particularly difficult problem since it is considered to have a very low infectious dose, and is also able to withstand low pH. Fortunately some intervention strategies have been devised to improve the safety of beef. Examples include whole carcase treatment with hot water or pressurised steam followed by a wash using hot lactic acid or acetic acid solution. Alternatively, low to medium dose irradiation of packaged beef products can be used.

Pasteurisation of milk effectively eliminates the hazard from milk and dairy products, but instances have occurred where there has been pasteurisation failure (Upton and Coia, 1994) and there are still many unpasteurised products marketed. This makes the production of clean milk, i.e. with low *E. coli* counts, of great importance.

Environmental contamination can be reduced by not allowing animals to graze on grass to which the public will have considerable access. Water supplies can be protected by add-in chlorine (Kaneko, 1998), though there are many private supplies in many parts of the world and chlorination may not always be practical. It is

important also to reduce the suspended solids to less than 5 mg/l for the process to be effective. Spray treatments of contaminated lettuce using 20 ppm chlorine, followed by rinsing later, were effective in removing VTEC O157 (Beuchat, 1999). Similar methods can be used for apples etc.

The control of VTEC O157 in animals

There is still a great need to attempt to reduce the faecal shedding from animals as it is difficult to assess the risk of contamination, impractical to disinfect every food product and impossible to exclude people from contaminated environments.

Hancock *et al.* (1998) discussed six possible control strategies for cattle:

1. Traceback and eradication

Since VTEC O157 is probably on most farms at some time this would be impractical.

2. Preslaughter testing of all cattle

This is flawed because the organisms are likely to survive on the hide. It would be more logical to test groups of animals and reject the whole group if any positives are found. It is likely, however, that too many groups would be rejected to make this a practical proposition. In the UK, at least one supermarket has used preslaughter testing, but the only action taken was to slaughter the positive groups of animals at the end of each day.

3. Other preslaughter measures

Efforts to reduce hide soiling are worthwhile and can be achieved by providing adequate bedding. In the UK many cattle are clipped but this is a dangerous operation without the use of a good set of stocks. Commonly, food is withheld before transport to avoid punctures of a full gut and to reduce defecation during transport, but there is evidence that fasting animals may actually increase the shedding of VTEC O157 (Duncan, *et al.*, 2000).

4. Vaccination

This is dependent upon VTEC O157 colonising and initiating a predictable immune response. Since neither of these happen consistently, it is unlikely that a means of vaccination will be found in the near future.

5. Competitive inhibition

The theory is that competitive organisms will inhibit the build up of VTEC O157, especially in cattle moved into feedlots. Probiotics have been used in young calves with some success in a small trial (Zhao, *et al.*, 1998) but this method needs to be converted to field trials before it can be adequately assessed.

6. Niche engineering

This was defined as 'Modifying the environment to make an ecosystem less susceptible to sustaining a particular agent'. Hancock *et al.* (1998) believe that, by the management of feed and water troughs, the multiplication of VTEC O157 may be reduced.

Duncan *et al.* (2000) have also reviewed the potential for the control in farm animals. This author believes that, by identifying animal husbandry methods or feed additives, proliferation of the organism in the gut may be avoided. The effects of some antibacterial plant compounds on *E. coli* could lead to the development of novel control methods.

The dynamics of VTEC O157

Recent studies in Scotland have shown a steady increase, in the last decade, of the phage type 21/28 that was very rare in the early nineties. The proportion of VTEC O157 producing the different verocytotoxins varies geographically. Table 2.1 shows the differences between Scottish and Washington State studies, for example (Rice, *et al.*, 1999).

Table 2.1 Percentage of cattle isolates with genes encoding the verocytotoxins in two studies

	VT1	VT2	VT1 + VT2
Scotland	3.5	95	3.3
Washington State	0.5	33	66.5

It is fascinating to conjecture that, if the prevalence in cattle is so high and there is frequent human exposure, why are more humans not affected. There has been a suggestion that there are two distinct lineages of VTEC O157 in the USA. Jaehyoung

(1999) has described an octamer-based genome scanning system that identifies a population of VTEC O157 found in cattle, but not found in man.

Recent studies in Scotland have shown a significant decline in prevalence of VTEC O157 across three seasons (Chapter 5). Care must be taken not to extrapolate these findings without evidence, but it is possible that VTEC O157 will one day disappear.

Conclusion

It is likely that different populations of VTEC O157 exist in different parts of the world. Because climates vary, and animal husbandry systems and human eating practices change so much from place to place, concerted activity is necessary all around the world to unravel the ecology and combat this infection.

Acknowledgement

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Chapter 3: PILOT PREVALENCE STUDY (1991) AND FIRST ZOONOTIC LINKS ESTABLISHED IN SCOTLAND

Abstract

Verocytotoxin producing *Escherichia coli*, and in particular the stereotype O157, have been associated with haemorrhagic colitis in man and the potentially fatal condition, mostly of children, haemorrhagic uraemic syndrome (HUS). Scotland has the highest incidence in the UK and the Grampian region is now reporting the highest incidence in the world: more than ten isolations per 100,000 population were reported in 1994. Food poisoning from verocytotoxin producing *E. coli* is less common than with *Salmonella* and *Campylobacter* but the consequences can be severe. Since verocytotoxin producing *E. coli* O157 has been linked to cattle elsewhere preliminary research has been carried out in Scotland to establish the prevalence in animals and to try to establish if infection in man could be linked to a cattle source.

In a preliminary study faeces samples routinely submitted to SAC's eight laboratories throughout Scotland were screened for *E. coli* O157. A total of 13 out of 5237 (0.25%) cattle samples yielded verocytotoxin producing *E. coli* O157, but there were no isolates from other species.

In the second part of the study the more sensitive technique of immunomagnetic separation was used to detect the organism in cattle epidemiologically linked to human cases. In ten out of nineteen incidents investigated an organism indistinguishable from the human isolate was isolated from cattle.

In one incident infection to humans from cattle was from improperly pasteurised or contaminated pasteurised milk. In two incidents raw milk could have been the source

but in seven incidents direct contact with cattle or contamination of food by cattle handlers was suspected.

This work was made possible by a very good working relationship between consultants in public health, medicine, environmental health officers, veterinary laboratories and the reference laboratories. Funding was from the Scottish Office Agriculture, Environment and Fisheries Department. There is a need to further investigate the role of cattle in this potentially fatal condition. The veterinary profession require the knowledge to adequately advise their clients.

Introduction

Verocytotoxin-producing *Escherichia coli* (VTEC) O157 was first recognised as an important human pathogen more than a decade ago (Riley, *et al.*, 1983). Although other food poisoning agents such as *Salmonella* and *Campylobacter* are more common, persons infected with *E. coli* O157 have a greater chance of severe illness. The agent is not invasive but adheres to mucosal cells of the intestine and destroys the microvilli (O'Brien and Holmes, 1987). The sequellae, haemorrhagic colitis, haemorrhagic uraemic syndrome and thrombotic thrombocytopenic purpura have been attributed to the verocytotoxin.

A bovine reservoir of verocytotoxin-producing *Escherichia coli* (VTEC) is now widely recognised. Isolations from healthy cattle have been reported in North America (Borczyk, *et al.*, 1987, Martin, *et al.*, 1986, Wilson, *et al.*, 1994) and from other countries, for example Germany (Montenegro, *et al.*, 1990). Isolates of VTEC O157 have been reported in the United Kingdom in abattoir surveys (Chapman, *et al.*, 1992, Chapman, *et al.*, 1989) and in farm animal surveys (Synge and Hopkins, 1992).

Only rarely however has the animal source of a human case of VTEC O157 infections been demonstrated (Chapman, *et al.*, 1993, Renwick, *et al.*, 1993, Synge, *et al.*, 1993). Outbreaks of VTEC O157 infection associated with eating undercooked "hamburgers" have been reported in North America (Belognia, *et al.*, 1991, Riley, *et al.*, 1983, Turney, *et al.*, 1994) and in the UK (Anonymous, 1991) along with other food borne outbreaks implicating dairy produce such as unpasteurized milk (Chapman, *et al.*, 1993) and yoghurt (Morgan, *et al.*, 1993). Other routes of transmission have been associated with contaminated drinking water supplies or handling or eating raw vegetables grown in ground on which farm manure had been spread (personal communication, J Curnow).

Materials and Methods

An initial survey was undertaken to establish the prevalence of *E. coli* O157 in livestock. Faeces samples and rectal swabs, from farm animals, companion animals and miscellaneous wild animals, which had been submitted to the eight (Scottish Agricultural College) Veterinary Services laboratories for routine diagnosis were included. Samples were plated on sorbitol MacConkey agar and the non-sorbitol fermenting colonies tested for O157 antigen using a commercial latex agglutination test (Unipath DR620M). Toxicity to verocells was confirmed using the method of (Chapman and Swift, 1984) and DNA hybridisation was used by reference laboratories at Aberdeen to confirm the presence of genes encoding verocytotxins (VT1 and VT2).

The second part of the study involved investigation of cattle herds which might have been the source of human infection. Epidemiological surveillance of VTEC infection in Scotland is based on the routine weekly reporting of isolates of *E. coli* by hospital

laboratories to the Scottish Centre for Infection and Environmental Health, Glasgow (Sharp, *et al.*, 1994). A detailed questionnaire form is completed for every human case in an attempt to identify possible sources of infection and individual herds were identified. Faeces samples were collected from 40 herds where epidemiological information suggested involvement of cattle.

The numbers of samples collected was sufficient to give a 90 per cent probability of isolating the organism assuming 5 per cent prevalence of detectable faecal carriage of *E. coli* O157. For this study the more sensitive isolation technique of immunomagnetic separation (Dynabeads 710.04, Dynal UK Ltd) was used. In addition to the phage typing and identification of verotoxin prodcution, isolates were compared at the reference laboratory by pulsefield gel electrophoresis. The Salmonella Reference Laboratory at Glasgow carried out plasmid profiling and fingerprinting of the plasmid nucleic acid.

Results

(a) Prevalence of E. coli O157

Table 3.1 shows the numbers of samples from the different species screened. Thirteen out of the fourteen bovine isolates produced verocytotoxin; all of these VT2 while two isolates also produced VT1. The isolates came from ten herds widely distributed throughout Scotland with one herd yielding three positive samples. The overall prevalence of VTEC O157 amongst cattle was 0.25%. *E. coli* O157 that does not produce verocytotoxin has been reported previously (Wray, *et al.*, 1993). The organism was not isolated from any other species.

Table 3.1 Samples examined and numbers yielding verocytotoxin-producing *E. coli* O157

Species		No of Samples	No. Yielding *E. coli* O157	No. % VT +VE
BOVINE	>2years	683	0	
	2 mth – 2 yrs	1721	2	2(0.12)
	< 2mth	2759	12	11 (0.40)
	Not known	74	0	
	TOTALS	5237	14	13 (0.25)
PORCINE		515	1	0(0)
OVINE		1542	0	
BIRDS	Poultry	392	0	
	Other	198	0	
DOGS		505	0	
CATS		181	0	
HORSES		127	0	
GOATS		104	0	
OTHER		156	0	
TOTALS		8957	15	13(0.15)

A higher proportion of isolations were from calves under two months of age than from older cattle. The majority of the samples submitted were from animals with diarrhoea but it is not possible to tell from the data if diarrhoea was correlated with VTEC O157 although in three instances where VTEC O157 was isolated from scouring calves no recognised pathogen was detected. Four different phage type strains were encountered viz PT32 (54%), PT49 (31%), PT1 (8%) and RDNC* (8%). All these phage types have occurred in man. In Scotland during the period of the study PT32 was not encountered among human cases while PT2 and PT49 accounted for 72% of human isolates. On following-up visits to six farms verocytotoxin-producing *E. coli* O157 was again isolated in two herds but the phage type was not always the same as for the first isolate.

(b) Investigation of Zoonoses

The study was more revealing than expected. In ten out of nineteen human incidents investigated *E. coli* O157, indistinguishable from a human case, was isolated from

cattle. Features of the isolates such as the verocytotoxins produced, the phage type and the fingerprint of the genome by pulse field and the plasmid profile corresponded in all these cases. In four herds VTEC O157 of a different type was isolated and in a further 36 herds no VTEC O157 was isolated..

Phage type 2 was encountered in seven incidents while phage types 49, 54 and 28 were each seen once only. The first link established between cattle and man was in Orkney (Synge, *et al.*, 1993). VTEC O157 had been isolated from a child with diarrhoea and medical investigation noted that there was a farm adjacent to the child's house. The child had visited the farm and the family pet dogs roamed freely through the cattle sheds and dung heap. On visiting the farm eighty four samples were collected and all were screened for VTEC O157. One sample from a faecal pat yielded the organism. Typing of the organism showed it to be indistinguishable from the human isolate. Both isolates were VT1 negative and VT2 positive, both were phage type 49, both had the unusual plasmid profile described as 90b:4 and the genomes of the isolates were indistinguishable by pulse field get electrophoresis.

Incident No. 3 was a dairy herd in Ayrshire at which the 53 year old farmer's wife regularly fed calves and drank raw milk. She developed severe bloody diarrhoea requiring admission to hospital. Incident No. 6 involved a suckler herd in Borders Region, where an eight month old child was regularly exposed to a cattle environment when he was taken daily in a pushchair to the cow shed where his parents worked. Following the onset of bloody diarrhoea the child developed haemolytic uraemic syndrome.

Incident No. 9 was a major epidemiological investigation following a major community outbreak in Lothian associated with a milk processing plant. (Upton and Coia, 1994). Cattle on eighteen dairy farms (herd nos. 9 to 26) supplying the plant

were investigated. In only one of these (herd no. 26) was VTEC O157 isolated. The organism was recovered from 16 of the 35 faecal samples examined and was indistinguishable from the human isolates.

The complexity of the situations that can be encountered was demonstrated by incident No.10. Following bloody diarrhoea in a 15 month old child in Dumfriesshire who had been admitted to hospital, three herds adjacent to the child's home (27-29, two dairy and one suckler) were investigated. In two herds (27 and 28) VTEC O157 of matching phage and verotoxin type to the human case was isolated. In one of these herds (28) and a third herd (29) VTEC O157 of a different type was also isolated. There was considerable regular contact with cattle faeces in the environment surrounding the house.

In addition to isolations from cattle faeces VTEC O157 was isolated from a hard cheese on two occasions that was epidemiologically linked to a human outbreak. The organism was not isolated from the milk used to make the cheese or from the cows that produced the milk.

The possible methods of spread of infection were investigated in each case and where cattle were implicated are summarised in table 3.2.

Table 3.2 Incidents Related to Possible Sources of Infection

No of Incidents	Source
1	Improperly pasteurised milk
2	Raw milk on direct contact
3	Direct contact or contamination of food by persons handling the cattle

Discussion

The initial prevalence study showed only 0.25% cattle samples positive for verocytotoxin producing *E. coli* (VTEC) O157. VTEC O157 was however shown to be present in thirteen out of forty herds and 5% of samples in the study using the more sensitive technique of immunomagnetic separation, which is claimed to be able to detect 100 *E. coli* O157 in a background flora of $>10^6$ organisms per ml. The herds in this study were selected on suspicion of being epidemiologically linked so the sample is biased., To get the true prevalence further work is required studying herds of cattle selected randomly. The results suggest however that *E. coli* O157 may be found in cattle frequently although in very low numbers. It appears however that only low numbers are required to cause human infection and the use of very sensitive detection techniques are essential if studying animals.

VTEC O157 was not found in any species other than cattle. Since this work however sheep have been implicated as carriers (Chapman and Siddons, 1996) and a recent study has linked the isolation from sheep with a human case on the basis of an indistinguishable isolate being obtained on the same basis as in the cattle studies described in this paper (personal communication D. Gray). VTEC O157 has recently been isolated from deer sharing the same pasture as cattle (Rice, *et al.*, 1995). In April 1996 four out of eight goats were shown to be carriers in an investigation following the death of a child from HUS (This thesis, Chapter 8, Table 8.2, Incident No.26).

The hypothesis that cattle may be the source of infection to man is supported by the results of sampling cattle epidemiologically linked to human cases. The risks of drinking raw cows milk or from failures in pasteurization are clear. The risks of contamination of other food stuff by cattle faeces and direct contact between cattle

and very young children should be emphasised. The paper on which this chapter is based (Synge and Hopkins 1996) then stated "It should not be assumed that the usual method of infection is from cattle; human to human spread is probably the most common route." Thinking has moved on and indeed cattle and sheep and their faeces are considered to be the principle source of sporadic human infection (see chapters 8 & 9).

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Chapter 4: FACTORS INFLUENCING THE SHEDDING OF VEROCYTOTOXIN-PRODUCING *ESCHERICHIA COLI* O157 BY BEEF SUSKLER COWS (A Longitudinal Study)

Summary

A study was designed to investigate management factors that might influence the shedding of verocytotoxin producing *Escherichia coli* (VTEC) O157 by beef cows in Scotland, where there is a particularly high rate of human infection. Thirty-two herds were visited at least monthly over approximately one-year for collection of fresh faecal pat samples and information on management factors. The faecal pat samples were tested for VTEC O157 by established culture and immunomagnetic separation methods. Questionnaires were completed at the monthly visits to record management factors. Data were analysed using both univariate and multi-factor (GLMM) analysis. Changes in the number of cows in a group, dogs, wild geese, housing, and the feeding of draff (distillers' grains) were statistically significant as risk factors. The event of calving appeared to reduce the likelihood of shedding. Any effects of weaning or turnout were not statistically significant. It appears that the rate of shedding of VTEC O157 is influenced by several factors but possibly the most important of these are the circumstances of animals being housed, or, when outside, the presence of wild geese.

Introduction

Escherichia coli O157 is now recognised as an important agent of human disease with world-wide distribution. There are approximately 200 cases of *E. coli* O157 infection in man reported annually in Scotland, where the rate per unit population is

consistently four times higher than in England and Wales (Smith, *et al.*, 1998). Haemolytic Uraemic Syndrome in the UK is associated most commonly with verocytotoxin-producing *E. coli* (VTEC) O157. While outbreaks are often food or water related, recent case control studies have indicated the importance of direct contact with animals as an important risk factor for sporadic cases (Locking, *et al.*, 2001, O'Brien, *et al.*, 2001). It is well known that cattle can be a reservoir of the organism. There is a mass of scientific literature on VTEC O157 in livestock, some of which is highlighted in a recent review (Synge, 2000). However, little is known about the factors that influence the shedding of VTEC O157 in cattle.

The objective of this study was to investigate management factors that might influence the shedding of VTEC O157 in beef suckler cow herds. VTEC O157 represent the highest risk to humans, and hence the study monitored the absence or presence of these bacteria in cattle faeces. Although age has an effect on the shedding of VTEC O157 (Synge, 2000), this study focuses on the adult beef suckler cow, despite the fact that various authors have suggested that calves are more likely than adults to be shedding the organism, e.g. in Australia (Cobbold and Desmarchelier, 2000), in USA (Hancock, *et al.*, 1994, Hancock, *et al.*, 1997), in the Netherlands (Heuvelink, *et al.*, 1998), and in the UK (Mechie, *et al.*, 1997, Synge, 2000). Effort was concentrated on suckler beef cows as these animals remain on farms for considerable periods of time, and therefore long-term data can be collected. In addition, they may serve as an important reservoir of infection, passing VTEC O157 to their offspring that subsequently enter the food chain. Laegreid *et al.* (Laegreid, *et al.*, 1999) showed that the widespread infection of beef calves at weaning was the result of infection prior to entry into the feedlots.

Previous studies have shown that the shedding of *E. coli* O157 is typically characterised by short duration, recurrent episodes which may indicate repeated exposure of animals to some source of this agent (Besser, *et al.*, 1997). Sources that have been hypothesised to be important include persistently shedding individual cattle, other persistent animal reservoirs, and environmental and food-borne sources. VTEC O157 has been isolated from sheep (Chapman and Siddons, 1996), goats (Chapman, 2000), wild deer (Rice, *et al.*, 1995), horses (Trevena, *et al.*, 1996), dogs (Trevena, *et al.*, 1996), geese (Smith, *et al.*, 1998), seagulls (Wallace, *et al.*, 1997) and pigs (Heuvelink, *et al.*, 1999). Animal reservoirs have been reviewed (Synge, 2000).

The organism has been shown to survive in bovine faeces for at least 99 days (Bolton, *et al.*, 1999). Hancock *et al.* (Hancock, *et al.*, 1994) implicated the spreading of cattle slurry on pastureland as a risk factor for the shedding of *E. coli* O157. Swerdlow *et al.* (Swerdlow, *et al.*, 1992) found sewage contamination of pasture lands or of drinking water supplies to be a source of infection which could result in subsequent spread to crops, animals and man. It has been suggested that diet may influence the shedding of *E. coli* O157 but many of these results are contradictory (Synge, 2000). Until a consensus is reached, diet cannot be overlooked as a potential risk factor. In addition to the already mentioned hypotheses this study examines the effect of events such as calving, weaning, housing and turnout on the risk of shedding. These events often involve transport, change in feed or other stressors that may be important in the shedding of *E. coli* O157. For example cattle can pass *E. coli* O157 from one to another (Synge, 2000). Transmission may be easier between housed animals kept at higher densities and hence in closer proximity.

All the above factors are examined in this study, which seeks associations between them and the shedding of VTEC O157. To the authors' knowledge, no intensive study has been carried out to examine the potential risk factors for shedding in beef-suckler cows. A Canadian study in seven dairy herds (Rahn, *et al.*, 1997) showed that shedding in dairy cattle was transient. A longitudinal study of a dairy herd (Mechie, *et al.*, 1997), and previous work in Scotland (Synge, 2000), reported seasonal incidence of shedding, but no attempts were made to explain these or assess if trends were statistically significant. A study involving 91 dairy farms in the USA (Garber, *et al.*, 1999) between February and July, showed cattle more likely to shed the organism after 1st May, but no explanation could be given for this phenomenon. More knowledge in this area could lead to the alteration of management practices to try to reduce the shedding of the organism, and therefore contribute to a lessening in the risk to human health.

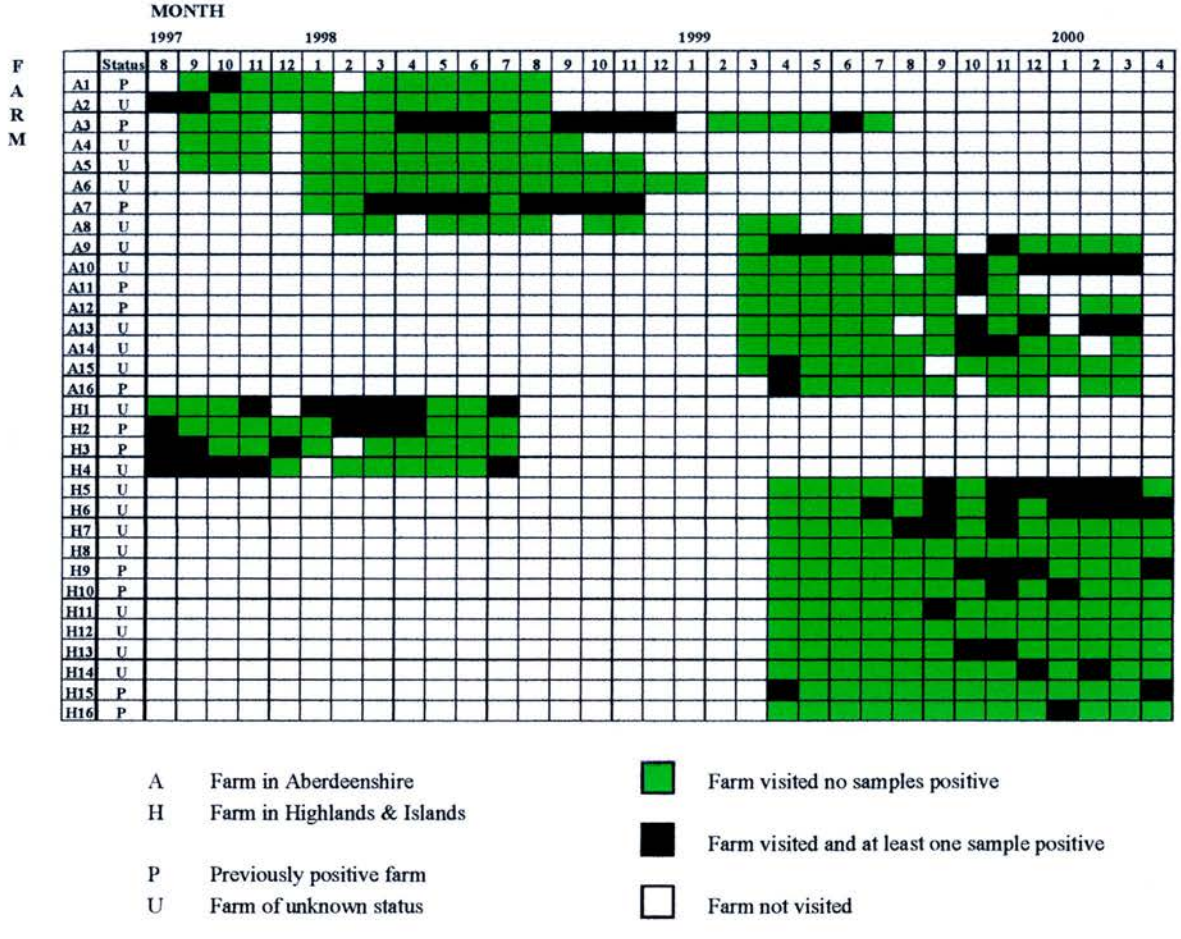
Materials and Methods

(a) Study Plan

Between August 1997 and April 2000 thirty-two farms in the north of Scotland were visited. The herds were not selected randomly, but on the basis of accessability. Twelve farms were known to have had a prior history of shedding before the start of the study. The status of the remaining twenty farms was unknown. Sixteen farms were in Aberdeenshire, and sixteen in the Highlands and Islands. Each farm was visited approximately monthly over a 12-month period with the exception of farm A3, which was sampled over a 23-month period. Farm A3 was known to be positive and was the subject of investigation because of a case of human infection with VTEC

O157. The farms were not all sampled concurrently. The first four farms in the Highlands and Islands and the first eight in Aberdeenshire were sampled between August, 1997 and January, 1999 while the remainder were sampled between March 1999 and April, 2000 (Figure 4.1). Calving was seasonal and generally confined to a maximum three month period. In the majority of herds this was February to April, in which case weaning was prior to housing and took place in September. In herds where calving took place in the summer or autumn weaning took place in the spring, before turnout, i.e. calves were approximately six months old at weaning.

Figure 4.1 Pattern of shedding in herds sampled monthly



Field Procedures

On each farm an isolated group of beef suckler cows were identified for sampling. The size of the groups ranged from 9 to 100, depending on the farm. This group was followed for the remainder of the study. Once the study began the farms were visited approximately monthly, although some farms were visited more frequently when events such as calving, weaning, housing, or turnout occurred. At each visit a farm management questionnaire was completed and faecal samples were taken and returned to the laboratory for analysis.

(a) Faecal Pat Sampling

The number of samples collected at each visit was determined using criteria that had been developed for a prevalence study (Synge, *et al.*, 2001). In summary, sample sizes were generated based on a model of within-herd prevalence assuming that 2% of herds would contain shedding animals. The shedding patterns on positive farms would be similar to those seen in data from farms previously investigated following human infection, varying around 10%. From this model the number of samples required to give an adequate probability of detecting that a herd contained cattle which were currently shedding was calculated. This power was set at 80%, a biologically acceptable value. For examples in a group of 20 cows, 17 samples were collected, with 30 cows 20 samples and 50 cows 23 samples were collected.

Samples were collected from fresh faecal pats into sterile plastic containers. These were tested on the same day except for samples from the two most remote farms, which were posted to the laboratory and tested within a week of sampling.

(b) Farm Management Questionnaire

There were five operators who collected samples over the course of the study. All received detailed information on the sampling criteria and methodology. Farm personnel were questioned on the following topics: feed, use of fertilisers, water supply, the presence of animals and the timing of events such as calving, housing, weaning and turnout (Table 4.1).

Laboratory Procedures

(a) Isolation

Three SAC disease surveillance centres at Thurso, Aberdeen and Inverness carried out direct culture on sorbitol MacConkey agar containing cefixime and tellurite (CT-SMAC). In addition 1g faeces was added to 20ml buffered peptone water and incubated for 6h at 37°C prior to immuno-magnetic separation (IMS) with O157 antibody coated beads followed by culture on to CT-SMAC. CT-SMAC plates were incubated at 37°C for 18 to 24 hours. Non-sorbitol fermenting colonies were selected and tested for agglutination with *E. coli* O157 latex reagent. The IMS method employed was similar to that described by Chapman (Chapman, *et al.*, 1994) but leaving out the antibiotics in the enrichment broth (Synge and Hopkins, 1996). The justification for this modification of the technique has been described previously (Synge, *et al.*, 1998).

(b) Typing

The Scottish *E. coli* Reference Laboratory carried out confirmatory tests of all isolates as *E. coli* O157, phage typing (Khakria, *et al.*, 1990) and examination for the verocytotoxin genes VT1 and VT2 using a multiplex PCR (Pollard, *et al.*, 1990). In

addition 332 isolates were tested for the *eae* gene which encodes for enterocyte attachment and effacement (Louie, *et al.*, 1994).

Statistical Analysis

(a) Case Definition

If VTEC O157 was isolated by either method from any animal in a group on a particular day the group was defined as positive on that occasion for the purpose of analysis.

(b) Farm Management Questionnaire

Data from all of the visits were recorded. The majority of variables were coded as present or absent on a given visit with the exception of the dynamic events: calving, weaning, bringing in, and turnout, which were recorded as having occurred soon before the sampling occasion. Where a sample was taken within the 14 days after the event occurred, the indicator variable was coded as present. Such criteria enabled the variables to be standardised across all farms. A variable for housed was also used in the model to differentiate between groups of animals that were currently housed and those that were grazing. In addition to the above the following quantitative variables were added to the database in preparation for multivariate analysis: the number of faecal samples taken, the number of positive faecal samples and the total herd size. Indicator variables such as 'were there changes in diet?' or 'were there changes in the number of suckler cows in the group since the last sample?' were also created.

Table 4.1 Factors investigated in the farm management questionnaire

Category	Specific Factors
Food	
Fodder	Hay, Pit Silage, Baled Silage, Straw, Root Crops
Concentrates	Home Concentrates: Barley & Other; Bought In Concentrates: Draff, Dark Grains, Cobs, Nuts, Other
Other	Minerals
Fertilisers	Organic Manure, Slurry, Human Sewage Sludge
Water supply	Mains, Private, Natural
Animals	Domestic (Sheep, Goats, Horses, Pigs, Poultry, Ducks, Geese, Cats, Dogs) and Wild (Gulls, Geese)
Events	Calving, Weaning, Housing, Turnout

(c) Statistical Methodology

Most univariate and all multi-factorial methods of analysis were carried out using SAS. Preliminary (univariate) analysis at the farm level was performed on all variables (Table 1) using odds ratios. Farms were divided into positive (VTEC O157 was detected in at least one sample) and negative (VTEC O157 was not detected in any sample) and each variable was recorded as being present (recorded on at least one visit) or absent (never recorded on the farm) and summed across farms to create a contingency table. Odds ratios were generated from the contingency tables with 90% confidence intervals while significance was tested using Fisher's Exact test. When examining the dynamic variables, such as calving or turnout, the most meaningful comparison is between the shedding status of farms before and after the occurrence of the event. The numbers of cases where farms switched from one shedding class to the other and where they remained in the same shedding class were recorded for each event

(Figure 4.2), as were the number of switches or no switches that took place in the absence of the events. The null hypothesis that these pairs of switching rates were equal (i.e. that on balance the event neither encouraged nor discouraged shedding) was tested using Fisher's Exact test.

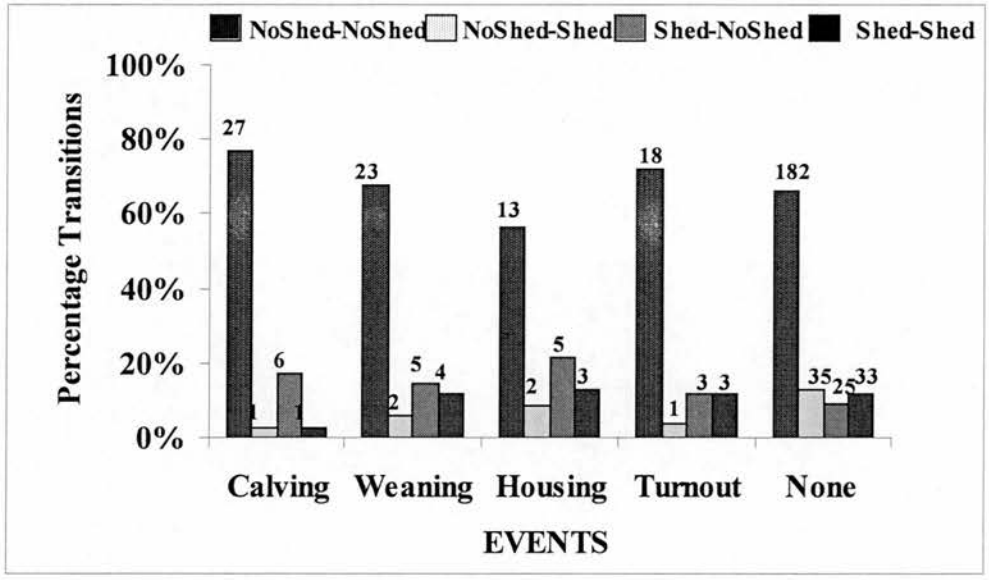
Multi-factorial analysis was performed by fitting a generalised linear mixed model (GLMM) (Brown and Prescott, 1999) to the number of positive samples from the total number of samples collected on each sampling occasion, using a binomial error distribution and logic link function. The GLMM allows the analysis to explicitly model both within and between farm variation. It also has advantages in handling data from observational studies such as this, where data are often unbalanced, both intentionally (focussing on events of interest) and unintentionally (where values are missing). The GLMM was fitted in SAS using the GLIMMIX macro, with Farm fitted as a random effect. Results were reported with p-values ≤ 0.10. Ideally, a model for the temporal autocorrelative structure of the within-farm variability would have been incorporated into the analysis. However, such models would not converge, possibly due to the highly unbalanced nature of the dataset. Using an alternative approach to compensate for autocorrelation, an indicator variable called "Previous Sample" was defined and fitted in the model. "Previous Sample" defines situations where the previous sample on a given farm included samples which tested positive for VTEC O157. It is reasonable to assume that a farm sample is more likely to be positive if even one of the samples from that farm on the previous visit was positive.

Figure 4.2 Prevalence of combinations of shedding patterns of VTEC O157 when evaluated before and after calving, weaning, housing, turnout and null events.

No Shed = not shedding; Shed = shedding.

For example the category NoShed-Shed summarises the number of occasions on which no shedding was initially present, but was observed in a set of faeces samples collected within 14 days of the specified event.

The figure above each bar represents the number of herd observations counted in a category, as defined with respect to the associated event.



The GLMM was initially fitted with 34 factors and variables and these were reduced using a backward stepwise elimination strategy until all that remained had p-values less than or equal to 0.10. Some variables had been removed prior to analysis as they were either not present on any farms or were present on most farms. Such uniform effects would cause the model convergence to fail, and give rise to meaningless parameter estimates. There was evidence of interactions between several of the

factors, and these interaction terms were included in the final model. Diagnostics were performed, and plots of residuals and farm-level random effects examined, confirming the goodness-of-fit of the model, while the stability of the model was assessed by determining the response of each variable to the removal of each factor.

Results

Laboratory Results

(a) Isolation

A total of 9256 faeces samples were processed by IMS and from these there were 392 positive for VTEC O157, giving an overall prevalence of 4.2% samples positive. The majority (7818) of samples were also subjected to direct culture but only five were positive by this method. Of 420 *E. coli* isolates collected 417 (99.3%) were confirmed to be *E. coli* O157.

(b) Typing

Of the 417 *E. coli* O157 isolates, 25 (6%) were found to be VTEC negative (had no VT genes) and were removed from subsequent analysis. Three hundred and fifty-seven (91%) of the isolates contained the VT2 gene only, thirty-four (8.7%) contained VT1 and VT2, but only one isolate (0.3%) contained VT1 only. All of the VTEC O157 tested were *eae* positive.

Field Results

(a) Farm-Level Patterns

Of the twenty farms that were of unknown status at the onset of the study, fourteen (70%) tested positive for VTEC O157 in at least one sample on at least one occasion (Figure 4.1). On seven out of the total thirty-two farms no VTEC O157 was isolated

at any point in the study. Among positive farms, sets of positive samples were isolated on between one and eight sampling occasions. The majority (92%) of positive farms, however, exhibited shedding for less than five months during the study (Figure 1). The longest consecutive period of shedding was a five-month block seen on farm H5. On most of the farms, however, shedding was detected in blocks of one or two months which could be separated by non-shedding blocks of anything between one and eleven consecutive months.

Table 4.2 Significant associations between factors on positive and negative *E. coli* O157 farms. Values represent counts of the number of farms. Sample size is in brackets.

	Positive	Negative	Odds Ratios (90% CI)
Housed	22 (25)	1 (7)	44.0 (5.7 – 340)***
Home Concentrates: Barley	18 (27)	1 (7)	15.4 (2.26 – 105.4)**
Cats	19 (25)	2 (7)	7.9 (1.64 – 38.3)**
Dogs	20 (25)	3 (7)	5.3 (1.19 – 23.9)*

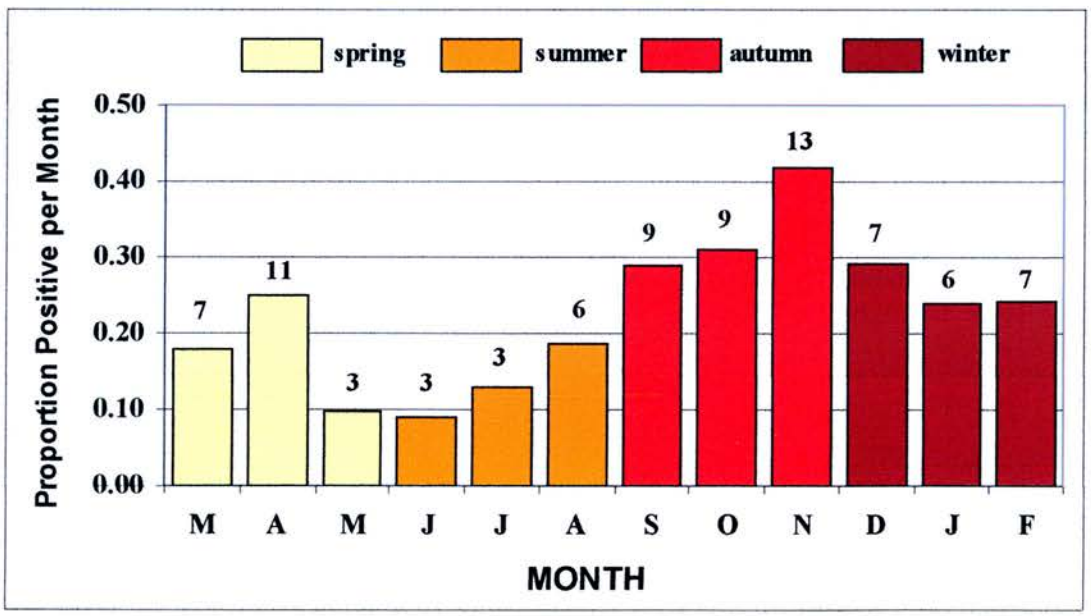
*p<0.1; **p<0.05; ***p<0.001

Univariate analysis showed no effect of farms using fertiliser or spreading manure, the feeding of forage crops or the type of water supply. However, there were significant statistical associations (p<0.001) between shedding and animals being housed (Table 4.2). Only one of the seven farms that were negative ever reported animals being housed for a period of longer than four days. Besides farms that housed animals, farms that fed home grown barley concentrate (p<0.05) or had cats (p<0.05) or dogs (p<0.10) were also more associated with shedding (Table 4.2). A small proportion of farms showed a change in the presence or absence of shedding associated with the events of calving, weaning and change in housing (Figure 4.2).

These effects were only significant for calving, where positive farms which contained calving animals were more likely to convert to negative status at the subsequent observation (p=0.03); negative farms with calving animals were also less likely to subsequently convert, although this was not statistically significant (p=0.07). Positive farms containing weaning animals were more likely to have retained their status since the previous observation, but this was not formally statistically significant (p=0.07). The housing of animals was associated with an increased risk of negative farms having converted to positive, while turnout was associated with an increased chance of positive farms having converted to negative, but neither of these effects were close to statistical significance. There was a significant seasonal effect with shedding being high in the autumn and low in the summer (χ^2_3 =13.88, p=0.003) (Figure 4.3).

Figure 4.3 Seasonal shedding of VTEC O157

The numbers above each bar indicate the numbers of herds with at least one cow shedding in any month



(b) Within and Between Farm Patterns

There was variation with respect to time in most explanatory variables at the within-farm level. This variation is likely to be important given the equally variable nature of the shedding of VTEC O157 at the within-farm level. Of the 34 variables that were considered for inclusion in the model, only four were significant as main effects (change in number of cows, pigs, dogs, wild geese); a further four variables were only significant as interactions with other variables (season, draff (a bought-in concentrate - distillers' grains), housed, and bringing in (Table 4.3). Bringing in has to be modelled as an interaction with housed since it is nested within this other factor. The indicator variable "previous sample" was significant (P = 0.0001) suggesting that there is temporal correlation in the data from individual farms. The inclusion of this variable dampens the significance of the other variables in the model and should allow a more meaningful interpretation of results. Several possible effects at the farm level are believed to be highly confounded. Even after fitting farm as a random effect, the residual deviance suggested that the data was somewhat overdispersed, but this is not unexpected from this type of epidemiological data.

Table 4.3 Odds Ratios and 95% CI for estimated effects in the GLMM analysis.

Variable	Estimated Effect	SE	Odds Ratio ψ	95% CI ϒ	P
Main Effects:					
Change in number of cows	0.82	0.240	2.3	1.42 – 3.65	0.0007
Pigs	-1.86	0.911	0.2	0.03 – 0.93	0.04
Dogs	2.15	0.543	8.5	2.95 – 24.8	0.0002
Wild Geese	1.39	0.589	4.02	1.27 – 12.7	0.0001
Interactions:					
Effect of Housed and Wild Geese:	3.54	1.05	34.4	4.42 – 267	0.005*
Effect of Wild Geese:					
(a) if Unhoused	3.29	0.820	27.0	5.42 – 134	0.004*
(b) if Housed	*0.52*	*0.643*	*1.7*	*0.48 – 5.9*	*1.00**
Effect of Housed:					
(a) if No Wild Geese	3.02	0.866	20.5	3.76 – 112	0.003*
(b) if Wild Geese present	*0.24*	*1.40*	*1.3*	*0.08 – 19.6*	*1.00**
Given Housed animals:					
(a) effect of Bringing in	-1.68	0.492	0.2	0.07 – 0.49	0.0007
(b) effect of Draff	1.23	0.463	3.4	1.38 – 8.47	0.04*
Effect of Wild Geese present:	3.33	0.910	27.9	4.69 – 166	0.008*
(a) if Summer	2.78	0.823	16.1	3.21 – 80.7	0.02*
(b) if Winter:					
Effect of no Wild Geese present:	1.76	0.407	5.8	2.62 – 12.9	0.0005*
(a) Autumn versus Spring					

ψ: odds ratio = exp(estimated effect)

ϒ: 95% confidence interval = exp(estimated effect ± 1.96*SE)

*: values adjusted using the Bonferroni correction

Further hypothesis testing of parameter estimates was conducted on the significant variables in order to determine the nature of any significant main effects and interactions. Where more than one comparison was made with respect to an effect, a Bonferroni correction was applied. Table 3 lists the odds ratios and 95% confidence intervals for the significant effects in the GLMM analysis. The following variables were associated as main effects with a risk of higher shedding ($p<0.10$; odds ratios >1): change in the number of suckler cows in the study group, the presence of dogs and the presence of wild geese (Table 4.3). By contrast, shedding appears to be lower when pigs are present on the farm. The Bringing in by Housed interaction was highly significant, representing an apparent protection factor. There were also significant interactions among the following variables: wild geese, season, housed and draff. Draff was found to interact with housed in a rational manner: only housed animals exhibited higher shedding rates while eating draff ($p=0.04$). There was no significant difference in shedding rates among grazing animals related to the feeding of draff. In order to examine the interactions between the other three factors, multiple two-way interactions had to be generated because the model would not converge using three-way interactions because of the sparseness of the data matrix. Housing is a highly seasonal factor. Of the three two-way interactions that were entered into the model, two were significant: season by wild geese and housed by wild geese. Season by housed was not significant.

It is difficult to separate the effects of housing, season and wild geese in these data. If animals are grazing, the presence of wild geese on the farm is a significant risk factor

for shedding. Among housed animals, the effect of wild geese is minimal. On farms, which have no wild geese present, housing is a clear risk factor, although on farms with geese present, housing has no apparent extra effect. In both summer and winter, there was statistically significant evidence that the presence of wild geese increased the risk of shedding, although it should be noted that only two farms reported wild geese as being present during the summer. On farms without geese, it was possible to establish that shedding levels were significantly higher in autumn than in spring, once allowance had been made for Housed and other significant factors. This was the only seasonal difference that was not explained by some other seasonally variable factor.

Discussion

The herds for this study were not randomly selected; hence it is invalid to make inferences about prevalence levels. However, the status of twenty of the thirty-two farms sampled was unknown at the beginning of the study, and these can be used to obtain an estimate of the farm prevalence of *E. coli* O157. Of the twenty, fourteen (70%) were positive at some stage. While a majority of farms were positive on at least one occasion, positive samples were detected only in a minority of visits (22%) (Figure 1). This figure closely matches the farm level prevalence (23%) found in a concurrent study of beef finishing cattle (Synge, *et al.*, 2001). This indicates a highly significant risk for persons coming into contact with beef cows or their faeces. Case control studies (Locking, *et al.*, 2001, O'Brien, *et al.*, 2001) have indeed suggested that direct contact with cattle or cattle faeces are important risk factors, at least for sporadic cases of *E. coli* O157 in man.

The observed shedding of *E. coli* O157 in this study was transient in nature. Similar observations have been made in other studies (Besser, *et al.*, 1997, Rahn, *et al.*, 1997). In an experimental study, inoculated calves shed the organism intermittently up to 58 days and cows up to 44 days, but on the majority of sampling days the organism was not detected (Wray, *et al.*, 2000). Besser *et al.* (Besser, *et al.*, 1997), found considerable variability in the excretion of *E. coli* O157 by cattle, noting that negative herds can change status suddenly and dramatically.

The vast majority of *E. coli* O157 isolated were verocytotoxin-producing and all those tested had *eae* genes. It has to be assumed, until proven otherwise, that all these isolates are potential human pathogens though there may be a subset of organisms found in cattle that are less likely to be human pathogens (Kim, *et al.*, 1999). Reports from the USA suggest a different proportional balance of VT types and combinations (Rice, *et al.*, 1999).

One of the key objectives in this study was to test for association between management events and shedding of VTEC O157. However when considering the power of this study to detect statistical associations for such shedding it must be remembered that, although this was a larger and more complex study than any previously reported, it generated a relatively small data set at the between-farm level. The results obtained must be interpreted with caution and should be interpreted as indicating possible risk factors for the shedding of VTEC O157 and used to develop further epidemiological hypotheses that can be tested in future studies. Conversely, smaller statistical effects may have been missed by this study.

No association was confirmed between shedding and housing or turnout in the univariate analysis, although it should be noted that housing was associated with relatively more farms becoming positive and turnout with relatively more farms becoming negative. The multi-factor model for shedding found strong effects due to housed and bringing in. Housed was, in general, associated with an increased shedding rate, but relative to this higher baseline, bringing in was associated with a lower shedding rate. This apparent contradiction may arise from other factors, which come into play when animals are inside e.g. close confinement (high population density) or possibly contaminated feed and water supplies. Rahn *et al.* (Rahn, *et al.*, 1997), found feed managers and water bowls had the highest rates of positivity for VTEC O157 suggesting they may play a role in animal to animal transmission. If such factors take some time to affect newly housed animals, those animals recently housed would indeed show a lower level of shedding, making the act of bringing in look like a protective factor. This would merely be an artefact of the inevitable nesting of 'recently housed' within housed. This does not preclude other possibilities, such as the change in ration at the time of housing being protective. A study in Switzerland of 67 cow-calf units showed increased shedding of VTEC in housed calves, but the calves were all older when at pasture than they were when housed (Busato, *et al.*, 1999), confounding age and housing effects. In a study of 36 dairy herds in USA, no association with housing was detected (Hancock, *et al.*, 1997). It is possible that variation in sanitary levels may generate the variation reported in these studies.

This study found some association between calving and shedding rates (rates dropping after calving was over) and weak evidence of the maintenance of shedding being associated with weaning. This evidence should be treated with some caution

since similar evidence was not found in the multi-factor analysis. Faecal shedding of *Salmonella spp.* is frequently associated with calving and the reason that there is not such a strong association with *E. coli* O157 may be related to a lack of invasion or colonisation in cattle. Given the transient nature of shedding it is possible that our study missed any association between some events and shedding. Sampling at more frequent intervals before and after such events would be necessary to provide sufficient coverage to establish these relationships. It should be noted that the power of the study to detect any association was limited given the relatively small number of calving and weaning events that were recorded.

Testing for associations between the shedding of VTEC O157 and the presence of other animals revealed that both dogs and pigs had significant effects. Cats had been significant in the univariate tests, but this could merely indicate that farms that kept cats all tended to exhibit some other risk factor at the farm level. The presence of dogs on the farm was significantly associated with increased shedding with dogs present on 80% of positive farms but only 43% of negative farms. It might be important to establish whether dogs can act as sources of this agent for cattle. Circumstantial evidence exists where dogs may have carried VTEC O157 from cattle to humans or *vice versa* (Synge, *et al.*, 1993) and another unpublished incident observed by the same author. There is a reported case where an indistinguishable VTEC O157 was isolated from a child and a dog but again it is not certain which was infected first (Trevena, *et al.*, 1996).

Unlike dogs, pigs were not a risk factor for the shedding of VTEC O157. However, the presence of pigs appeared to be protective. Although Heuvelink *et al.* (Heuvelink,

et al., 1999) isolated VTEC O157 from Dutch slaughter pigs others (Chapman, *et al.*, 1997) found in a small local survey that pigs were not a major reservoir of infection. *E. coli* O157 isolates from pigs are frequently not verocytotoxin producing (Wray, *et al.*, 1993). Unfortunately this study contained very few pig farms with which to explore the nature of this association. It is likely that the presence of pigs *per se* is not important, rather that pig farms may tend to implement management practices that favour reduced shedding. This is corroborated by the fact that there was no evidence of the presence of pigs being protective in the univariate analysis, i.e., that the pig factor only becomes statistically significant in the analysis in conjunction with other epidemiological factors. More information would have to be gathered under controlled conditions before the full epidemiology of the shedding of VTEC O157 in cattle and other domestic animals can be properly identified.

Another risk factor was found among the cattle themselves. As this was a field study conducted on working farms it was not always possible to maintain the integrity of the sampling groups. Changes in the number of cattle in the study group were recorded at each visit. These changes may influence levels of VTEC O157 shedding. Increases in numbers may lead to increased shedding when the cows that are introduced are actively shedding O157. A Canadian study in dairy cattle showed that open herds are more likely to shed VTEC O157 (Wilson, *et al.*, 1993) and a similar conclusion has been drawn from a concurrent Scottish prevalence study (Synge, *et al.*, 2001).

The GLMM analysis allows the exploration of more complex associations between risk factors. There appears to be a complicated inter-relationship between three of the risk factors analysed: season, housing and the presence of wild geese. Unfortunately

this study was not large enough to allow a full analysis of the interaction between all of these variables; however, there are strong indications that these important factors do influence the shedding of VTEC O157 by beef-suckler cows. These findings are consistent with the scenario where housed increases shedding, and the presence of wild geese increases shedding among exposed (grazing) animals, and it is coincidental that the increases in each case happen to be similar.

The presence of wild geese was a significant risk factor when considered in association with the season and housed variables. Previously, VTEC O157 has been isolated from domestic geese (Smith, *et al.*, 1998) and seagulls (Wallace, *et al.*, 1997), while large numbers of wild geese were reported as present on certain farms during the study (data not presented). The risk from wild geese, however, is not constant but mediated by management practices. Shedding is significantly higher when geese are present but only among grazing animals. On most farms the cattle graze in the spring and summer while the majority of animals are brought in in the autumn and winter months. Hence it is not surprising to see the apparent effect of wild geese varying by season, though it is surprising that the strongest (and statistically significant) effects are seen in summer and winter. The summer results should be treated with some caution, since they are driven by the experience of only two farms that reported wild geese in summer with concurrent high shedding levels. The winter results require careful interpretation, since the proportion of housed animals in the winter with geese group is much greater than that in the winter with no geese group. Cattle eating forage made from pastures on which there had been geese might explain the winter results.

Housed proves to be a significant risk factor in the GLMM analysis, both as a main effect and in interaction with bringing in and draff (distillers' grains). Housed animals fed draff were more likely to shed VTEC O157. Draff was never fed on any farm that proved negative for VTEC O157. There is evidence in the literature that some feed can support the growth of the organism; (Fenlon and Wilson, 2000) this study would suggest that the feeding of draff should be studied as a possible contributory factor in the shedding of O157.

VTEC O157 cases in man tend to be more common in the summer months (Douglas and Kurien, 1997). The literature generally describes a corresponding peak of shedding by cattle in the summer e.g. in the USA (Hancock, *et al.*, 1997) or in the Netherlands (Heuvelink, *et al.*, 1998). However if the published data is examined carefully the UK shedding peak is in the spring or late summer/autumn (Chapman, *et al.*, 1997, Mechie, *et al.*, 1997, Synge, 2000). It is the interaction between seasonally variable factors (wild geese, bringing in, housed and BICD feeding) that is likely to be responsible for the different seasonal patterns observed in the univariate and multi-factor analyses.

In this study the seasonal pattern from univariate analysis (in decreasing order of shedding) is autumn > winter > spring > summer. However, after the GLMM has allowed for the effects of other significant explanatory factors, the resulting seasonal pattern (in decreasing order of shedding) is summer > autumn > winter > spring, with all but one of these seasonal differences not being statistically significant. The initial pattern is likely the result of a three-way interaction between wild geese, season and housing. The univariate analysis indicated that autumn and winter were the seasons

with the highest shedding. This is the time when the cows tend to be housed and hence are affected by this risk factor for the shedding of VTEC O157. In the multi-factor analysis summer was associated with the highest unexplained shedding, but this effect is very variable and not significantly higher than the other seasons. Focussing only on farms with no wild geese, there is some evidence that the shedding in autumn is significantly higher than that seen in spring (Table 4.3). This, of course, is in line with earlier reports. This is a complex situation which needs more exploration. In general, purely seasonal patterns should be viewed with caution in the future, with attention rather being focused on other, possibly more informative, management factors.

It appears that the rate of shedding of VTEC O157 is influenced by several factors, possibly the most important of these being the state of being housed and, when outside, the presence of wild geese. A more detailed understanding of the biological basis for the observed variability in shedding will be critical to make use of such information in the development of on-farm control measures.

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Chapter 5: THE PREVALENCE OF VEROCYTOTOXIN-PRODUCING *ESCHEICHIA COLI* O157 IN FINISHING BEEF CATTLE IN SCOTLAND AND FACTORS INFLUENCING SHEDDING

Summary

The prevalence of verocytotoxin-producing *Escherichia coli* O157 in 12-30 month old beef finishing cattle in Scotland was determined using 1 gram faeces samples enriched in buffered peptone water without antibiotics, followed by immunomagnetic separation (IMS) and isolation on sorbitol MacConkey agar with cefixime and tellurite supplement (CT-SMAC). The decision not to use antibiotics in the enricment medium was reached following a comparative pilot study. A validated questionnaire was used to collect information that could be associated with the samples. Generalised Linear Models and Generalised Linear Mixed Models were used to identify risk factors for shedding both between and within groups.

Prevalence levels were calculated with 95% confidence intervals as follows:
7.9% (6.5%, 9.6%) of animals sampled were shedding VTEC O157.
22.8% (19.6%, 26.3%) of farms had at least one animal shedding in the group sampled.

Increased probability of a group containing a shedding animal was associated with larger numbers of finishing cattle, and the presence of pigs on the farm. Farms that spread slurry on grazing land were more likely to have shedding animals, while those which spread manure were at lower risk. Groups with older animals were less likely to be identified as positive. There were no significant regional differences in group

shedding probabilities, but the proportion of positive groups dropped over two successive years of the study. Higher mean levels of shedding in positive groups were associated with animals being housed rather than at pasture. Farms with animals at pasture had lower mean prevalences where water was supplied from a natural source, as had farms with higher numbers of finishing cattle. There remained unexplained variability in mean prevalence levels on positive farms in different areas of Scotland.

Introduction and Bacground

A very large outbreak of disease associated with verocytotoxin-producing *Escherichia coli* (VTEC) O157 occurred in central Scotland in 1996 (Ahmed and Donaghy, 1998) in which twenty people died. Following this an expert group recommended that it was important to establish the prevalence of VTEC O157 in Scottish cattle (Pennington, 1997). This paper summarises the findings of the subsequent study (1998–2000), which was designed to find both the prevalence and to elucidate the epidemiology. This remains the largest prevalence study for VTEC O157 ever carried out and forms the basis of much of the subsequent work carried out under the Wellcome Foundation International Partnership Research Award in Veterinary Epidemiology (IPRAVE). The completed analysis is presented here for the first time, where the findings are discussed in the light of a concurrent longitudinal study (Synge, *et al.*, 2003).

Verocytotoxin-producing *E. coli* (VTEC) O157 is an important cause of diarrhoea in man and in some cases serious consequences follow, such as haemorrhagic colitis, haemolytic uraemic syndrome or thrombocytopenia, which may be fatal. There are approximately 200 human cases reported annually in Scotland and the rate per unit

population is approximately three times that in England & Wales (Smith, *et al.*, 1998). Although human infection may arise from person to person contact or from consumption of food contaminated by asymptomatic human carriers, it is accepted that several species of animal (cattle, sheep, horses, goats, dogs and geese) also carry the organism. Frequently, primary human infection can be attributed to contamination of the environment or the food chain from such animals. It was well known that cattle might act as a reservoir of infection (Synge and Hopkins, 1992) but the likely prevalence of shedding of the organism was unknown. There remains little understanding of factors that might influence shedding. A greater understanding of the epidemiology could lead to possible interventions at farm level to reduce the shedding of this hazardous organism.

Published information about the prevalence of faecal shedding of *E. coli* O157 in cattle populations shows wide variation. Table 5.1 lists examples of reported prevalences from different classes of cattle in different countries. True comparisons are difficult because the execution of these studies has not been consistently rigorous and problems arise from the lack of uniformity of the study designs and the laboratory methods. Outcomes will have been affected by the populations from which samples are drawn; the sampling methodologies adopted; the timing of sampling; and the sensitivity of the tests used for screening. An early prevalence study in Scotland (Synge and Hopkins, 1996) using direct plating on sorbitol MacConkey agar found only 0.25% of bovine faeces samples submitted to the veterinary investigation laboratories positive for VTEC O157, whilst a study of cattle at the Sheffield abattoir, using similar methods, found 4% of cattle to be shedding the organism (Chapman, *et*

al., 1993). The introduction of immunomagnetic separation (IMS) as a more sensitive technique (Chapman, *et al.*, 1994) has probably generated more accurate estimates of prevalence, reducing the downwards bias of estimates from direct plating methods.

To provide a better estimate of shedding rates in Scottish cattle entering the food chain, a study was designed with the objective "to estimate the herd level prevalence of verocytotoxin-producing *E. coli* (VTEC) O157 shedding for fattening cattle in Scotland using IMS test on 1 gram faeces samples". As sub-objectives, the animal level prevalence for positive herds was estimated and the effect of a variety of potential risk factors on these prevalences were investigated.

Table 5.1 Estimates of *E. coli* O157 prevalence from the published literature

Country	Class of cattle	Isolation method	Prevalence	Reference	Year
Scotland	Diagnostic samples	Direct culture	0.25%	(Synge and Hopkins, 1996)	1993
Sheffield, UK	Abattoir	Direct culture	4%	(Chapman, *et al.*, 1993)	1993
England & Wales	Diagnostic samples	IMS	0.83%	(Richards, *et al.*, 1998)	1998
USA	Dairy cattle	Enrichment	0.28%	(Hancock, *et al.*, 1994)	1994
USA	Feedlot cattle	Direct culture	0.33%	(Hancock, *et al.*, 1994)	1994
USA	Calves	Enrichment	1.5%	(Zhao, *et al.*, 1995)	1995
Finland	Slaughter cattle	IMS	1.31%	(Lahti, *et al.*, 2001)	2001
Netherlands	Adult cattle	IMS	11.1%	(Heuvelink, *et al.*, 1996)	1996

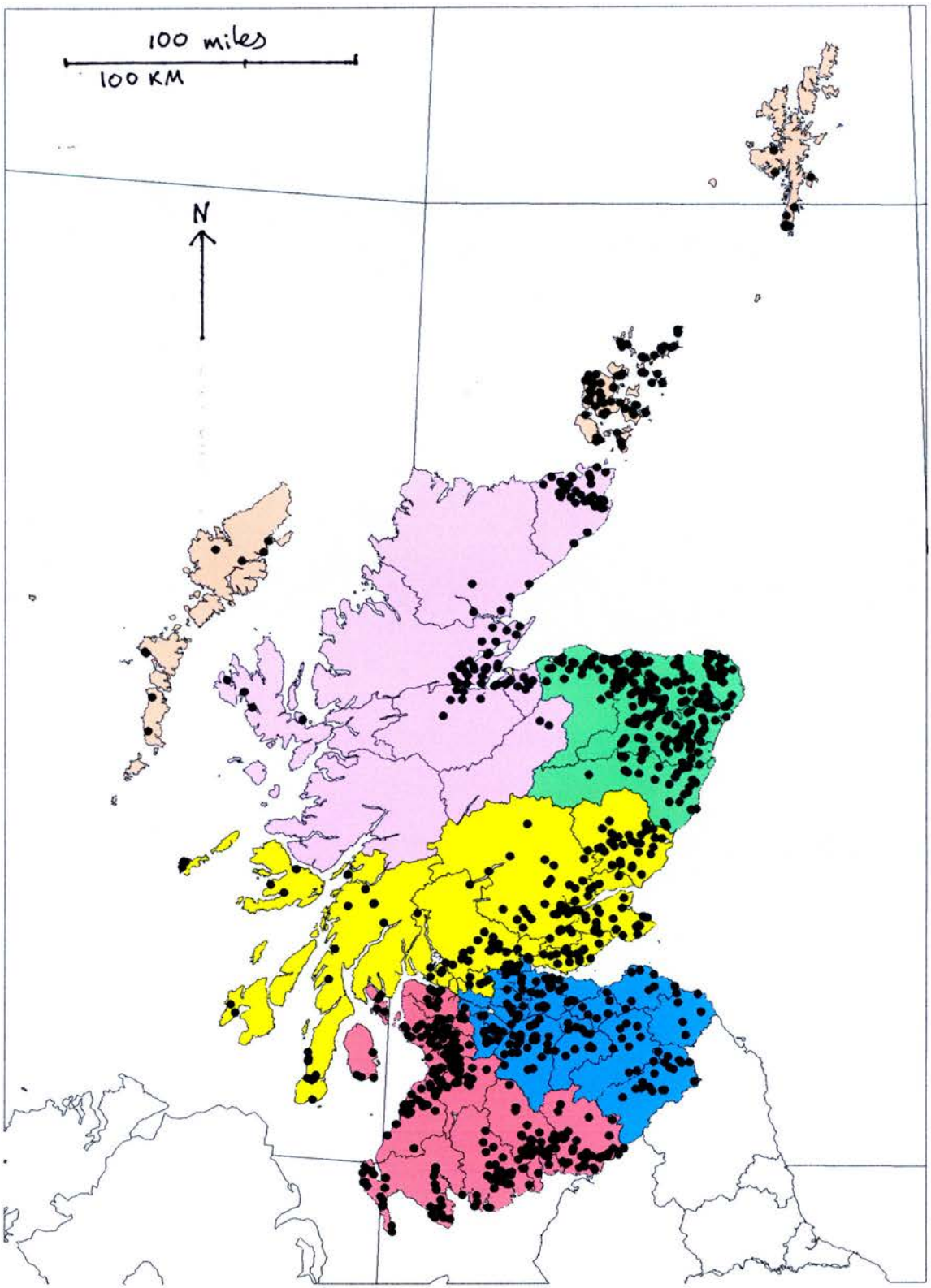


Figure 5.1 The six regions of Scotland used for the study. (brown = Islands, purple = Highlands, green = North East, yellow = Central, blue = South East and pink = South West. The dots represent the positions of the herds sampled.
(This work is based on data provided with the support of the ESRC and JISC and uses boundary material which is copyright of the Crown, and the Post Office. Source: The 1991 Census, Crown Copyright. ESRC purchase.)

Materials and Methods

Study Design

Herds likely to contain fattening cattle were selected randomly from throughout Scotland using a sampling frame defined by information in the Scottish Executive farm census. For the purpose of the study Scotland was divided into six regions: the five Animal Health Divisions with the Northern and Western Isles forming a separate region (Figure 5.1). The set of farms to be sampled were stratified by farm-management type and by region. Pilot data were used to estimate a distribution for the within-herd prevalence on positive farms, suggesting a mean within-farm animal prevalence of 10%. This distribution of prevalences was used to derive an on-farm sampling plan, relating the number of pats sampled to the number of animals in the sampling group, with the objective of identifying as positive 80% of groups containing at least one shedding animal. Assuming a herd prevalence of 2%, the number of herds to be sampled was specified as giving an 80% probability of the estimate of the farm prevalence being precise to a tolerance of +/-1%. On the basis of these assumptions, it was calculated that the study was unlikely to have an acceptable statistical power to detect anything other than major differences in prevalence caused by potential risk factors. Nevertheless, it was thought worthwhile to collect epidemiological information from the farms included in the survey. However, since the herd prevalence of shedding was much higher in practice, it proved possible to carry out an extensive investigation into risk factors with information collected from the farm management questionnaire. Ten farms were revisited and on this occasion all animals on the farm were sampled. These figures have not been included in the main dataset, but are presented in Table 5.3.

Field Procedures

Each region was sampled in turn on a weekly rotation, with sample herds being visited on a random basis within each region. Only cattle aged 12 to 30 months were sampled and on each farm the group of such animals nearest to slaughter was selected. At each visit a farm management questionnaire was completed and faecal samples were collected and returned to the laboratory for analysis.

(a) Faecal Pat Sampling

When collecting samples from faecal pats, no pat can be identified as coming from any specific animal. Having assumed that shedding animals do not defaecate at any increased rate relative to non-shedding animals, it is therefore assumed that sampling without replacement from the population of faecal pats is equivalent to sampling with replacement from the population of animals. It seems likely that this will be a reasonable approximation given a moderately sized population of faecal pats. Pilot data suggested a within-herd prevalence which was distributed as a beta distribution with a mean of approximately 10%. For a group of size N, a beta-binomial distribution was used to describe the number of positive animals in the herd, Y, with a binomial model giving the estimated probability of detecting at least one positive pat with a sample of size n as $1-\left(1-\frac{Y}{N}\right)^n$.

From this model the numbers of samples required to provide an 80% probability of detecting that a herd contains shedding cattle were calculated. Samples were collected from fresh faecal pats into sterile plastic universal containers. The samples were returned to the laboratory and tested within 48 hours.

(b) Farm Management Questionnaire

At each visit in addition to taking faecal samples, a farm management questionnaire was completed. There were two operators who collected samples over the course of the study. A detailed validation procedure was undertaken to validate the questionnaire and eliminate operator bias. Farm personnel were asked questions on the numbers of cattle on the farm and about the numbers of groups kept, their source and breed type. For housed cattle questions were asked regarding the type of housing and the management during the previous four weeks. This included the timing of housing or movement, the type of forage or concentrate fed and how the silage was made. For grazing animals questions were asked about movements of the group, applications of slurry or manure onto the fields and supplementary feeding. For all groups of animals questions were asked about the presence of other animals on the farm and the water supply. A full list of the factors investigated in the questionnaire is given in Table 5.2.

Table 5.2 Variates and Factors Collected by the Farm Questionnaire.

Abbreviation	Factor/Variable	Levels
Manage_O	Observed management type.	Breed, Dairy, Others, Mixed
Division	Animal Health Division, with one division divided into Highlands and Islands.	Central, Highlands, Islands, NE, SE, SW
Sam_Month	Month in which samples were collected.	January-December
Sample	Type of sampling scheme.	Faecal Pat, Rectal
Sam_Year	Year in which samples were collected.	1998, 1999, 2000
Sampler	Person carrying out sampling.	H, F (codes)
N_F_Cattle	Number of finishing cattle on farm.	Variate
FCattle	Number of finishing cattle, categorised into groups.	<50, 50-99, 100-199, 200+
N_Groups	Number of management groups of cattle on farm.	Variate
GroupsCat	Number of management groups, categorised into groups.	1, 2-5, 6-9, 10+
N_Sam_Gr	Number of finishing cattle in sampling group.	Variate
Min_Age	Minimum age of animals in sampling group.	Variate
Max_Age	Maximum age of animals in sampling group.	Variate
Source	Farm policy for replacement cattle.	Buy In, Breeding Only, Both
NewSource	Restructuring of 'Source' into open and closed farms.	Open, Closed
Breed	Breed of cattle in sampling group.	Beef (Suckler Beef), Dairy Beef, Dairy (Bull Beef), Combinations of these
Housed	Whether sampling group are housed or unhoused.	Housed, Unhoused
Housing	For housed animals only: type of housing.	Court/Straw Yard, Slats,

		Byre, Other
TDHouse	Number of months for which animals have been in current housed state.	Variate
Rec_Move	Whether or not the sampling group have been moved in the 4 weeks prior to sampling.	Yes, No
SupFeed	For unhoused animals only: whether the sampling group is fed supplements.	Yes, No
RecDFeed	Whether or not the sampling group have had a change in diet in the 4 weeks prior to sampling.	Yes, No
Forage	For housed animals only: whether the sampling group is fed forage.	Yes, No
Silage	For housed animals only: whether the sampling group is fed silage.	Yes, No
Concentrate	For housed animals only: whether the sampling group is fed concentrate.	Yes, No
Sil_Home	For housed animals fed silage only: whether the farm produces silage.	Yes, No
Sil_Manure	For housed animals fed farm-produced silage only: whether the farm spreads manure on the silage fields.	Yes, No
Sil_Slurry	For housed animals fed farm-produced silage only: whether the farm spreads slurry on the silage fields.	Yes, No
Sil_Sewage	For housed animals fed farm-produced silage only: whether the farm spreads sewage on the silage fields.	Yes, No
Sil_Geece	For housed animals fed farm-produced silage only: whether geese have been observed on the silage fields.	Yes, No
Sil_Gulls	For housed animals fed farm-produced silage only: whether gulls have been observed on the silage fields.	Yes, No
Hay	Whether the farm produces hay.	Yes, No
Hay_Manure	If the farm produces hay only: whether the farm spreads manure on the hay fields.	Yes, No
Hay_Slurry	If the farm produces hay only: whether the farm spreads slurry on the hay fields.	Yes, No
Hay_Sewage	If the farm produces hay only: whether the farm spreads sewage on the hay fields.	Yes, No
Hay_Geese	If the farm produces hay only: whether geese have been observed on the hay fields.	Yes, No
Hay_Gulls	If the farm produces hay only: whether gulls have been observed on the hay fields.	Yes, No
Grass_Manure	Whether the farm spreads manure on pasture.	Yes, No
Grass_Slurry	Whether the farm spreads slurry on pasture.	Yes, No
Grass_Sewage	Whether the farm spreads sewage on pasture.	Yes, No
Grass_Geece	Whether geese have been observed on pasture.	Yes, No
Grass_Gulls	Whether gulls have been observed on pasture.	Yes, No
N_Cattle	Number of cattle on farm (other than finishing group).	Variate
Cattle	Number of cattle on farm (other than finishing group), categorised into a factor.	<100, 100-499, 500-899, 900+
N_Sheep	Number of sheep on farm.	Variate
Sheep	Absence/presence of sheep on farm.	Yes, No
N_Goats	Number of goats on farm.	Variate
Goats	Absence/presence of goats on farm.	Yes, No
N_Horses	Number of horses on farm.	Variate
N_Pigs	Number of pigs on farm.	Variate
Pigs	Absence/presence of pigs on farm.	Yes, No
N_Chickens	Number of chickens on farm.	Variate
Chickens	Absence/presence of chickens on farm.	Yes, No
N_Deer	Number of deer on farm.	Variate
Deer	Absence/presence of deer on farm.	Yes, No
Mains	Whether sampling group is watered with a mains supply.	Yes, No

Private	Whether sampling group is watered with a private supply.	Yes, No
Natural	Whether sampling group is watered with a natural supply.	Yes, No
WaterCon	Whether water have been contaminated within the 12 months prior to sampling.	Yes, No
WaterCT	Possible sources of contamination.	Animals Upstream, Septic Tank, Midden, Combinations of these
Want2Know	Whether farmer wishes to know results of sampling.	Yes, No
Visit2	Whether farmer is willing to have a further set of samples collected.	Yes, No
LabOperator	Lab operator responsible for assaying faeces samples.	S, D, H (codes)
BeefonDairy	Whether farm is classed as a dairy farm with suckler beef cattle.	Yes, No

Laboratory Procedures

(a) Isolation

E. coli O157 isolation was carried out at the SAC centre in Inverness using category 3 facilities. All staff were validated in the techniques to rule out any inter-operator bias. All samples were tested by immunomagnetic separation (IMS) for *E. coli* O157 using a standard methodology which was selected following a blind laboratory trial (Foster, *et al.*, 2003). Briefly, 1 g of faeces was suspended in 20 ml of buffered peptone water (BPW) (Oxoid, Basingstoke, Hants., UK) without antibiotic supplement and incubated at 37°C for 6 h. Following incubation, 1ml was dispensed into a 1.5 ml sterile micro-centrifuge tube (Alpha Laboratories, Eastleigh, Hants., UK) containing 20 µl of *E. coli* O157 coated magnetic beads (Dyna-beads anti-*E. coli* O157, Dynal, Oslo). The tubes were shaken manually to disperse the beads through the broth, before putting them on to a rotamixer for 30 minutes. The sample vials were then loaded on to an immunomagnetic separator (Dynal) and left for 3 mins during which time the beads became attached to the side of the vial. The broth was removed using a sterile 1 ml pasteur pipette and replaced with 1 ml of 10 mM phosphate buffered saline at pH 7.4 with 0.05 % Tween 20 (Sigma Diagnostics, Poole, Dorset, UK) (PBST). The tubes were removed from the magnet and shaken to re-suspend the

beads and placed back on the magnet for a further 3 min and the PBST removed. This process was repeated a further two times after which the beads were resuspended with two drops of PBST from a pasteur pipette which was inoculated on to CT-SMAC plates and incubated at 37°C for 18-24 h. CT-SMAC plates were examined for pale non-sorbitol fermenting colonies which were spot-inoculated on to Chromocult agar (Merck, Poole, Dorset, UK) and incubated at 37°C for 18-24 h. Colonies that appeared pink on Chromocult agar were tested for agglutination with *E. coli* O157 latex (Oxoid). A sub-culture from each latex positive sample was sent for confirmation and phage typing to the Scottish *E. coli* Reference Laboratory, Department of Medical Microbiology, Foresterhill, Aberdeen. Each isolate was also stored on Cryobank beads (Mast Diagnostics) at -80°C.

(b) Typing

All isolates were first confirmed as *E. coli* O157. They were phage typed using standard methods (Khakria, *et al.*, 1990) and examined for genes encoding the production of the verocytotoxins VT1 and VT2 and the eae gene which encodes for enterocyte attachment and effacement using a multiplex PCR (Louie, *et al.*, 1994, Pollard, *et al.*, 1990).

Data Management

Data were entered into an Excel (Microsoft) spreadsheet. Data for each individual field were summarised and checked against the valid range for entries. A sample (10%) of records were checked against the original questionnaires. A 0.4% error rate was detected, with a 95% confidence interval of (0.2%, 0.6%), indicating that, on average, the data entry process had been reliable. These errors were, however, concentrated in a small number of fields, and these fields were checked against the

original questionnaire for all records. No entry errors were found in the recorded numbers of VT+ samples. All identified errors in the data were corrected prior to analysis.

Statistical Analysis

If VTEC O157 was isolated from any animal in a group, the group was defined as positive. The proportion of animals in any group shedding VTEC O157 summarised the level of shedding on that farm. Summary tables were compiled comparing the isolation rates, phage types and VT encoding genes. Graphs and charts were used in exploratory data analyses.

The prevalence data were initially analysed using a Generalised Linear Model, but the poor fit given by this approach necessitated the use of an alternative methodology. The data are treated as being the outcome of a mixture distribution, where a proportion p_{neg} of the population are defined as negative farms and will always return a zero number of positive samples. In the positive population, the between farm variability is modelled as a beta distribution, taking parameters a and b, while the sampling distribution of the faecal pat sampling process is taken to be binomial. A small number of farms were sampled where pats could be assigned to individual animals, and hence the process is equivalent to rectal sampling. The sampling distribution of this process is taken to be hypergeometric. No positive samples were collected from this latter group. Hence, where N is the number of animal in the group, n is the number of samples collected, and x is the observed number of positives, the distribution of x is taken to be:

$$P(X=0)=\begin{cases} p_{neg}+(1-p_{neg})\dfrac{\Gamma(n+b)\Gamma(a+b)}{\Gamma(a+b+n)\Gamma(b)} & \textit{under faecal pat sampling} \\ p_{neg}+(1-p_{neg})\displaystyle\sum_{i=0}^{N-n}\binom{N-n}{i}\frac{\Gamma(i+a)\Gamma(n-i+b)\Gamma(a+b)}{\Gamma(a+b+N)\Gamma(a)\Gamma(b)} & \textit{under rectal sampling} \end{cases}$$

$$P(X=x,x>0)=\begin{cases}(1-p_{neg})\binom{n}{x}\dfrac{\Gamma(x+a)\Gamma(n-x+b)\Gamma(a+b)}{\Gamma(a+b+n)\Gamma(a)\Gamma(b)} & \textit{under faecal pat sampling}\end{cases}$$

Hence, although two different sampling distributions are involved, they are based on the same underlying parameters and can be incorporated into the same likelihood. The log-likelihood is maximised with respect to a, b and p_{neg}. The distribution of number of cattle in the sampling groups is modelled as a log-normal distribution. Assuming no relationship between size of group and the variability in prevalence summarised in the beta distribution, the beta-binomial model was used to estimate the fraction of groups which contained at least one shedding animal.

Confidence intervals for the prevalences were generated using the properties of the log-likelihood function in the vicinity of the maximum. Because of the strong negative correlation between p_{neg} and a and b, p_{neg} was set equal to the maximum likelihood estimate. Marginal confidence intervals for the mean prevalences were then generated from the profile log-likelihood by identifying the maximum and minimum values of the prevalences on the boundary of the confidence region specified by the chi-squared approximation to the profile log-likelihood ratio. Two variables were assumed unfixed, so the confidence interval was based on two degrees of freedom.

Confidence intervals for shedding prevalences on individual farms were calculated using the exact confidence interval for binomial proportions (Armitage, *et al.*, 2002)

for data from rectal samples from a complete group, and a variation of this approach to allow for excess sampling variability for those data from faecal pat samples.

Risk factors were analysed using the Generalised Linear Model (GLM) and Generalised Linear Mixed Model (GLMM) procedures in Genstat. The fitting of a single model to the dataset was not possible, since the large number of negative observations, coupled with a subset of observed farms with high shedding levels, gave rise to an extremely badly fitting model. This reflects the bimodal nature of the dataset, where many farms are likely to be consistently negative for shedding. The analysis therefore proceeded by the analysis of the subset of the data with non-zero shedding results, and an analysis of the entire dataset, restructured to identify absence/presence of shedding at the farm level. The GLM structure is appropriate for both of these analyses since the data are Binomial in form, and the use of random effects in this model ensures that the highly unbalanced nature of the data does not give rise to biased estimates of epidemiological effects. However, the use of mixed models is highly computer intensive, and so standard GLMs were used for the univariate aspects of the analysis described below. GLMs and GLMMs were fitted with a Binomial distribution and a logit link function in each case.

GLMs were fitted with an overdispersion parameter to model excess variability in the data where appropriate. When fitting a GLMM, Farm, Veterinary Practice (Vet) and County were all examined as possible random effects. County and Vet were not found to be useful in explaining any of the variability of the data, so Farm was used as the sole random effect. The dispersion parameter was set equal to 1. Other epidemiological variables were fitted as fixed effects.

GLM analyses were initially carried out in a univariate basis, analysing each factor or variate in turn. When analysing the shedding levels in positive groups, it was concluded that much of the variability in the data was explained by a specific factor. A further set of restricted multivariate analyses were therefore carried out, fitting a GLM model incorporating this factor in interaction with each candidate factor or variate in turn. The statistical significance of each variate in the GLM is assessed using the *F*-distribution approximation to the ratio of the change in deviance to the residual deviance.

Any factor or variate with a p-value less than or equal to 0.1 in any of these analyses was carried forward for more detailed analysis. Groups of highly correlated candidate factors were assessed for goodness of fit, and those factors giving good fit were then reviewed using a forward stepwise selection algorithm and the Akaike information criterion to select candidates for inclusion in the multivariate model. A forward stepwise selection algorithm was used to review whether any previously rejected factors or variates should be added to the draft multifactor model. The resulting draft multifactor model was then fitted using a GLMM. The statistical significance of each variate in the GLMM is assessed using the chi-square approximation for the Wald test. Finally, the study design factors Animal Health Division and Farm Type were included in the multivariate models, as were descriptive factors defining the time of sampling. The estimated effects associated with these descriptive factors were compared with those from the associated univariate analyses. All *p*-values in the final analysis are the results of two-sided tests, even where the biological hypothesis might

have allowed the definition of a sensible one-sided test. This is in line with standard statistical practice.

The mean prevalences for subsets of farms associated with different risk factors were derived from the GLMM using an adaptation of the method proposed by (Condon, *et al.*, 2004) to allow for the effect of random effects on the non-transformed means. Rather than using Monte Carlo integration as these authors did, Latin Hypercube sampling (LHS) was used to generate a less variable estimate.

Results

Prevalence

A total of 14,856 samples were collected from 952 herds. Of these 1,296 were positive for *E. coli* O157 and 1,231 positive for verocytotoxin producing *E. coli* (VTEC) O157. These VT-positive samples were sourced from 207 farms. Hence, the raw figures indicate that 21.7% of groups sampled contained shedding animals, and that the animal level prevalence is 8.3%. Using the beta-binomial model, it is estimated

- that the prevalence of VTEC O157 shedding in finishing cattle is 7.9% with a 95% confidence interval of (6.5%, 9.6%)
- that 22.8% of finishing groups contained at least one positive shedding animal, with a 95% confidence interval of (19.6%, 26.3%).

The point-estimate and confidence interval for the group prevalence are both slightly higher than the raw estimates given earlier, since the latter figures incorporate an

adjustment for farms with low shedding rates being wrongly misclassified as negative due to sampling variability.

Within herd prevalence census

Of the ten farms that were revisited, six had been positive previously. It was frequently impossible to identify a group of animals on the second visit with characteristics (age and management status) analogous to those specified in the initial visit. One initially negative farm was assessed as positive after a gap of six weeks, and one initially positive farm was assessed as negative following a seven-month gap; however, no analogous animals were examined on the second visit. Two farms exhibited statistically significant changes in within-group prevalence as illustrated by Table 5.3. Note that in several cases the 95% confidence intervals are extremely wide.

Table 5.3 Percentage of pats and cattle positive for VTEC O157 at successive sampling times on 10 farms, with associated 95% confidence intervals.

Farm	Pats Positive (Initial Visit*)	95% CI for %-age Cattle Positive• (Initial Visit*)	Cattle Positive (All Animals Follow-up Visit)	Cattle Positive (Analogous Animals* Follow-up Visit)	95% CI for %-age Cattle Positive (Analogous Animals* Follow-up Visit)	Comparison *p*-value
1	15/15 (100%)	(68%, 100%)	43/142 (30%)	40/75 (53%)	(41%, 65%)	**0.004**
2	16/18 (89%)	(55%, 99%)	0/123 (0%)	na	na	-
3	8/15 (53%)	(19%, 85%)	21/120 (18%)	8/30 (27%)	(12%, 46%)	0.18
4	1/7 (14%)	(1%, 74%)	20/93 (22%)	20/93 (22%)	(14%, 31%)	0.90
5	2/21 (10%)	(1%, 36%)	10/103 (10%)	na	na	-
6	1/14 (7%)	(0.3%, 46%)	5/240 (2%)	na	na	-
7	0/19 (0%)	(0%, 24%)	0/109 (0%)	0/20 (0%)	(0%, 17%)	1.00
8	0/11 (0%)	(0%, 43%)	0/106 (0%)	0/86 (0%)	(0%, 4%)	1.00
9	0/25 (0%)	(0%, 16%)	18/156 (12%)	18/38 (47%)	(31%, 64%)	**<0.001**
10	0/13 (0%)	(0%, 37%)	0/78 (0%)	0/15 (0%)	(0%, 22%)	1.00

*Animals sampled during initial visit were fattening cattle, aged 12-30 months. On the follow-up visit, when all animals were rectally sampled, only a subset of animals (if any) would meet this description. These animals are defined to be an analogous group for comparative purposes. •Confidence intervals based on pat samples incorporate additional pat-related sampling variability, and can therefore be interpreted as reflecting the properties of the cattle population.

Verocytotoxins

The vast majority (1168) of isolates expressed the VT2 gene only (94.9%, with a 95% confidence interval ranging from 93.5% to 96.0%). Three isolates, or 0.2% of the total (0.05%, 0.7%) expressed VT1 only while sixty (4.9% with a 95% confidence interval ranging from 3.7% to 6.2%) expressed both VT1 and VT2.

Enterocyte attachment and effacement

Genes encoding enterocyte attachment and effacement (eae) were detected in all isolates including those that were non-toxigenic, giving a 95% confidence interval for the true prevalence of (99.7%, 100%).

Phage type analysis

Table 5.4 shows the numbers and percentages of the different phage types exhibited amongst the *E. coli* O157 isolates that were verocytotoxin-producing or not verocytotoxin-producing. The vast majority of VTEC isolates were phage type 21/28.

Table 5.4 Numbers of each phage type isolated (%)

Phage Type	O157 VT-ve		O157 VT+ve	
2	21	(32)	181	(15)
4	10	(15)	74	(6)
8	0		56	(5)
14	1		12	(1)
21/28	7	(10)	722	(59)
24	0		5	
31	0		1	
32	11	(17)	145	(12)
54	7	(10)	2	
RDNC	8	(12)	33	(3)
Total	65		1231	

Multiple phage types on farms

Table 5.5 shows the occurrences of one or two phage types on farms. The top diagonal line of figures shows the number of farms where one phage type only was found. For example on 23 farms phage type 2 only was found and on 116 farms phage type 21/28 only was found. The other figures show the number of farms where two phage types were detected. For example on three farms phage types 2 and 21/28 were detected but on one farm phage types 2 and 31 were exhibited. Thirteen farms exhibited two phage types.

Table 5.5 Number of occurrences of two phage types on one farm

Phage	2	4	8	14	21/28	24	31	32	54	RDNC
2	23									
4	0	8								
8	0	0	8							
14	0	0	0	2						
21/28	3	2	0	1	116					
24	0	0	0	0	0	0				
31	1	0	0	0	0	0	0			
32	1	0	1	0	1	0	0	25		
54	0	0	0	0	1	0	0	0	0	
RDNC	1	0	0	0	1	0	0	0	0	8

Three farms exhibited three different phage types and one farm exhibited four different phage types including the only type 24 recorded.

Analysis of the absence/presence of shedding

The majority of groups (78%) had no shedding animal detected. The results of the univariate analysis are summarised in Table 5.6 Values are given for all factors or variates which gave rise to a p-value less than 0.1 in either univariate analysis, which were ultimately included in a multi-factor model, which are fundamentally

descriptive, or which summarise important aspects of field or laboratory practice. Terms rejected as giving rise to poorly fitting models are suppressed.

Table 5.6 Results of the univariate analysis: absence/presence of shedding. Factors with *p*-values less than 0.1 in the univariate analysis are given in bold type.

Factor/Variable	*p*-value	Comments
Manage_O	0.80	'Beef' and 'Others' farms have higher mean prevalences than 'Dairy'.
Division	0.16	'Highland' farms have lower mean prevalences than others.
Sam_Month	**0.06**	Lower mean prevalences in January and February. Anomalously low mean prevalences in April and June, anomalously high mean prevalence in November.
Sam_Year	**0.004**	Consistent drop in mean prevalence with time.
Sample	0.28	Lower mean prevalence for farms assessed using rectal samples.
Sampler	0.18	Farms with samples collected by 'F' have a higher mean prevalence than those with samples collected by 'H'.
LabOperator	**0.04**	Farms with samples assayed by 'S' had a lower mean prevalence than farms assayed by 'D' and 'H'.
FCattle	**<0.001**	Farms with between 50 and 199 finishing cattle have higher mean prevalences than those with fewer than 50 animals, the mean prevalence for farms with over 200 animals is higher still.
GroupsCat	**0.08**	More groups is associated with a higher mean prevalence.
N_Sam_Gr	**<0.001**	More animals in the sampling group is associated with a higher mean prevalence.
SamGrF	**<0.001**	Farms with fewer than 11 animals in the sampling group have lower mean prevalences than those with 11-28 animals. Farms with greater than 28 animals have still higher mean prevalences.
Max_Age	0.31	Higher maximum age associated with lower mean prevalence.
Source	**0.01**	Farms classed as 'Buy in' and 'Both' show higher mean prevalences than those classed as 'Breeding only'.
NewSource	**0.03**	Farms classed as 'Open' show higher mean prevalences than those classed as 'Closed'.
Breed	**0.03**	Farms with stock classed as 'B_D_DB' have higher mean prevalences than others. No consistent pattern.
Housed	0.64	Farms with Housed animals have higher mean prevalences.
Rec_Move	0.66	A recent move (within four weeks) is associated with a lower mean prevalence.
RecDFeed	0.69	A recent change in feed (within four weeks) is associated with a higher mean prevalence.
Sil_Home	0.83	Farms with housed animals which also produce silage have higher mean prevalences than all other farms.
Sil_Manure	0.68	Farms with housed animals which also spread manure on their silage fields have lower mean prevalences than all other farms.
Sil_Slurry	0.16	Farms with housed animals which also spread slurry on their silage fields have higher mean prevalences than all other farms.
Grass_Manure	**0.02**	Farms with unhoused animals which also report the use of manure on grass have a lower mean prevalence than all other farms.
Grass_Slurry	**<0.001**	Farms with unhoused animals which also report the use of slurry on grass have a higher mean prevalence than all other farms.
Cattle	**0.002**	Farms with fewer than 100 cattle have lower mean prevalences than those with more animals.
Sheep	0.42	Farms with sheep present have higher mean prevalences than those without sheep.
Pigs	**0.01**	Farms with pigs present have higher mean prevalences than those without pigs.
Deer	0.38	Farms with deer present have higher mean prevalences than those without deer.

BeefOnDairy	**0.02**	Farms classed as Dairy units but stocking 'Beef' breed animals have a higher mean prevalence than other farms.

Several factors summarise information about the number of cattle on the farm; these are highly correlated, and ultimately only SamGrF, Cattle and FCattle are included in the draft multifactor model. NewSource is derived from the Source factor, and is preferred in the later analysis. The Breed factor is reformulated to summarise only whether or not a farm stocks animals from the B_D_DB class (Breed2). There is clear evidence of temporal changes in prevalence, with evidence of a year-on-year drop in prevalence. This trend and the unbalanced nature of the dataset appear to explain the reported changes in monthly and seasonal prevalences. This also explains the apparent laboratory operator effect: the availability of different laboratory staff is confounded with time. Restricting attention only to the period when both members of staff were assaying samples, there is no statistically significant evidence ($p=0.77$) of any difference in the prevalences arising from their work. Using the stepwise procedures to evaluate candidate factors and variates, it is found that Breed2 lacks significance when other factors are included. This probably reflects the fact that the class of animal which the univariate model identified as higher prevalence only occurred on six farms in total. Cattle and NewSource lack significance when the cattle-related factors are included. The latter result can be explained by the observation that larger farms tend to buy in replacement cattle; only small operations will breed all the replacements required. Max_Age is added to the model, since it shows evidence of statistical significance in the model once more of the residual deviance is explained by the other factors. Hence, the factors FCattle, SamGrF, BeefOnDairy, Gra_Slurry, Gra_Manure and Pigs and the variate Max_Age are included in the Generalised Linear Mixed Model. Sampling Year and Sampling

Month are also included. Farm, County and Veterinary Practice are fitted as possible random effects, but only Farm proves statistically significant. The results of the multi-factor model are summarised in Table 5.7.

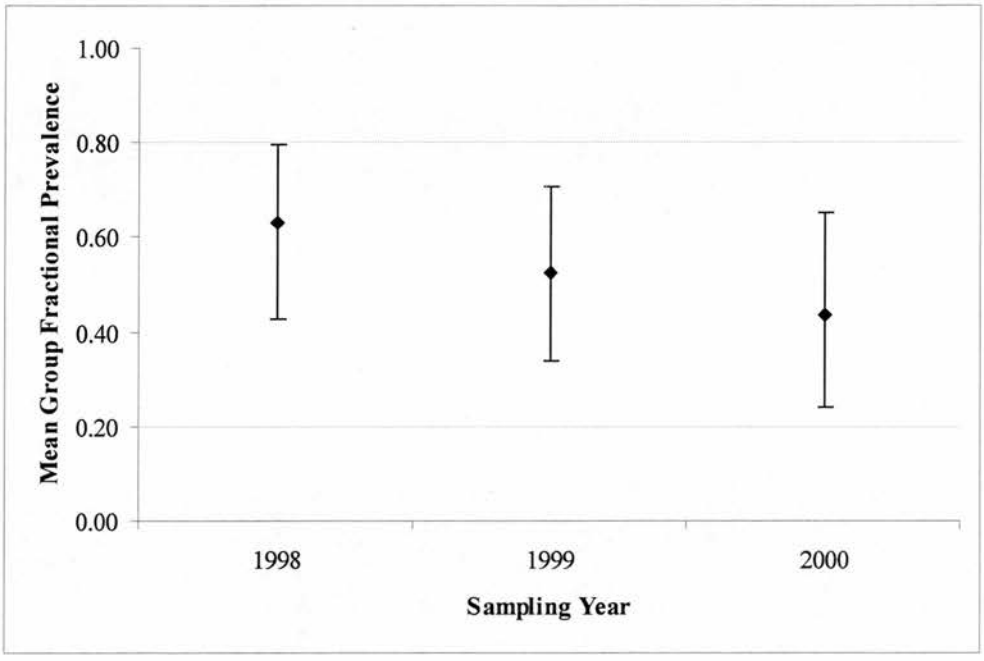
Table 5.7 Results of the multifactor analysis: absence/presence of shedding.

Factor/ Variable	Effect	Log Odds Ratio	se	*p*-value
Sampling Year	Allowing for the explanatory factors, farms sampled in year 1999 are at lower risk of being positive than those sampled in 1998.	-0.425	0.21	0.04
	Allowing for the explanatory factors, farms sampled in year 2000 are at lower risk of being positive than those sampled in 1999.	-0.371	0.26	0.15
	Allowing for the explanatory factors, farms sampled in year 2000 are at lower risk of being positive than those sampled in 1998.	-0.795	0.31	0.01
Sampling Month	A broad cyclical effect, with prevalence peaking in Summer and troughing in Winter. Anomalous changes in prevalences observed in a number of months, such as April, June and November.	Various	Various	0.02
Categorised Number of Animals in Sampling Group (SamGrF).	Farms with 12-28 animals are at a higher risk of being positive than those with less than 12 animals.	0.687	0.23	0.003
	Farms with greater than 28 animals are at a higher risk of being positive than those with 12-28 animals.	0.462	0.19	0.03
Categorised Number of Finishing Cattle (FCattle).	Farms with 50-199 animals are at a higher risk of being positive than those with 1-49 animals.	0.367	0.19	0.05
	Farms with more than 200 animals are at a higher risk of being positive than those with 50-199 animals.	0.614	0.30	0.04
Spreading of Slurry on Pasture	Considering only farms with animals at pasture, those which spread slurry are at a higher risk of being positive than those which do not.	1.205	0.32	<0.001
Spreading of Manure on Pasture	Considering only farms with animals at pasture, those which spread manure are at a lower risk of being positive than those which do not.	-1.155	0.36	0.001
Dairy Farms with Beef Cattle	Dairy farms with beef cattle are at a higher risk of being positive than other farms.	1.965	0.64	0.002
Presence of pigs on farm.	Farms with pigs are at a higher risk of being positive than those without pigs.	0.892	0.35	0.01
Maximum age of cattle in sampling group.	Higher maximum age is associated with a lower risk of the farm being positive.	-0.031	0.015	0.04

The factor SamGrF is correlated with the number of animals in the sampling group and hence with the number of samples collected from the group. Hence a positive relationship could be generated through the higher detection probability arising from a larger sample. Consideration of the data suggests that this is unlikely, but even if the result is discounted on this basis, the inclusion of FCattle in the multi-factor model even in the presence of the sampling group factor indicates that the size of enterprise remains a highly significant risk factor.

No statistically significant geographical or management system variability was observed in the analysis of the basic data, and nothing further became apparent following the fitting of the multi-factor model. By contrast, the basic data showed evidence of a long-term trend towards lower prevalences over the lifetime of the study, and this trend remained in the multi-factor model, unexplained by any of the proposed explanatory factors (Fig. 5.2). The basic data showed no significant evidence of any cyclicity by month or season, although various peculiarities were observable in the analysis. When included in a model with the full multi-factor model, the month effect is found to be significant. Most important in this respect are the effect of low mean prevalences in April and June, and a high mean prevalence in November. It is important to stress that 'pattern' matches that observed in the univariate model: the effect is not an artefact of a poorly fitting model. Hence it can be concluded that the farm level prevalences do vary with month, in a fashion which is not explained by the proposed explanatory factors.

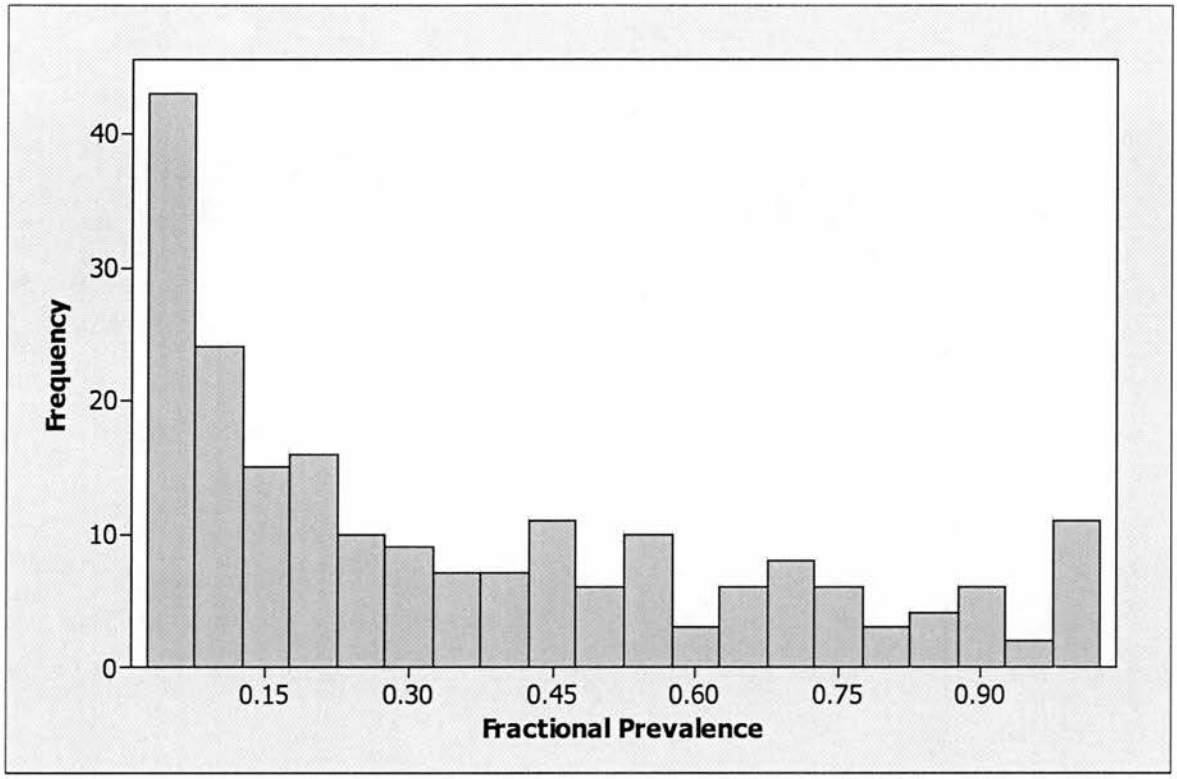
Figure 5.2 Estimated means and 95% confidence intervals for the group-level fractional prevalence in the years 1998-2000; values adjusted for other significant covariates and random effects in the multi-factor model.



Analysis of levels of shedding in positive groups

It was notable that most herds that were identified as containing at least one shedding animal had a low proportion of positive pats. However a spectrum of levels of shedding were seen and a number of herds appeared to have virtually all animals shedding (Fig. 5.3).

Figure 5.3 Histogram of proportions of faecal pats positive for VTEC O157 from positive groups.



The results of the univariate analyses indicated that housing status had an overwhelming effect on mean prevalence. Many of the factors listed in Appendix 1 are partially or wholly confounded with housing. Hence, rather than reporting the simple univariate results, it is more informative to present the results from fitting each factor and an interaction term, in turn, to a model already containing housing status. These restricted multivariate results are summarised in Table 5.8. Values are given for all factors or variates which gave rise to a p-value less than 0.1 in either univariate analysis, which were ultimately included in a multi-factor model, which are fundamentally descriptive, or which reflect aspects of field or laboratory practice. Terms rejected as giving rise to poorly fitting models are suppressed.

Table 5.8 Results of the restricted multivariate analysis: levels of shedding in positive groups. Factors with *p*-values less than 0.1 in the restricted multivariate analysis are given in bold type.

Factor/Variable	*p*-value	Comments
Manage_O	0.33	'Beef' farms have a higher mean prevalence and 'Others' a lower mean than 'Dairy' farms.
Division	**0.007**	'Highland' farms have a significantly higher mean prevalence than others.
Sam_Month	0.31	No apparent pattern in mean prevalence.
Sam_Year	0.23	No apparent pattern in mean prevalence.
Sample		Not fitted: all positive farms were faecal pat sampled.
Sampler	0.42	No apparent pattern in mean prevalence.
LabOperator	0.45	No apparent pattern in mean prevalence.
FCattle	**0.032**	The larger the group of cattle, the lower the mean prevalence.
GroupsCat	0.41	No apparent pattern in mean prevalence.
N_Sam_Gr	0.20	More housed animals in sampling groups is associated with lower mean prevalences, more unhoused associated with higher mean prevalences.
SamGrF	0.80	No apparent pattern in mean prevalence.
Max_Age	0.40	Higher maximum age associated with lower mean prevalence in unhoused farms, while in housed farms, the contrary is observed.
Source	**0.09**	Source significant in interaction with Housed factor. For unhoused animals, farms which 'Buy In' have lower mean prevalences, while for housed animals, farms which 'Buy In' have a higher mean.
Breed	0.67	No apparent pattern in mean prevalence.
Housed*	**<0.001**	Farms with housed animals have a much higher mean prevalence.
Rec_Move	**0.004**	Farms with housed animals which have been moved during the previous 4 weeks have a lower mean prevalence.
RecDFeed	**0.024**	Farms with housed animals which have had a change in feed during the previous 4 weeks have a lower mean prevalence.
Sil_Home	**0.04**	Farms which have housed animals and which produce their own silage have a lower mean prevalence than other farms with housed animals.
Sil_Manure	**0.047**	Farms which have housed animals and which spread manure on their silage fields have a lower mean prevalence than other farms with housed animals.
Sil_Slurry	**0.027**	Farms which have housed animals and which spread slurry on their silage fields have a lower mean prevalence than other farms with housed animals.
Grass_Manure	0.59	Farms which spread manure on their pasture and which have unhoused animals have a lower mean prevalence than all other types of farm.
Grass_Slurry	0.39	Farms which spread slurry on their pasture and which have unhoused animals have a lower mean prevalence than all other types of farm.
N_Cattle	**0.012**	In housed groups, the presence of more cattle is associated with a lower mean prevalence.
Cattle	0.18	No apparent pattern in mean prevalence: perhaps some evidence of lower mean prevalences in larger housed groups.
Sheep	0.10	Farms with sheep present have a lower mean prevalence than those without.
N_Goats	0.49	Different effects observed in housed and unhoused groups.
Goats	0.58	Different effects observed in housed and unhoused groups.
Pigs	0.38	In unhoused groups, the presence of pigs is associated with a lower mean prevalence, the opposite effect is observed in housed groups.
Deer	**0.036**	The presence of deer is associated with a higher mean prevalence;

		poorly fitting factor.
Natural	**0.03**	Unhoused animals with water supplied from a natural source had lower prevalences than unhoused animals supplied from mains or private supplies.
BeefonDairy	0.59	This class of farm exhibits a higher mean prevalence in housed groups, lower in unhoused.

*Results for Housed factor are for a univariate analysis.

A highly statistically significant seasonal effect was observed in the univariate analysis, with mean prevalence levels peaking in January while being relatively low between June and October. This pattern matches the management of housing in Scottish herds, and when the housing factor was included in the model, the temporal factors exhibited no statistically significant effects. Highly statistically significant differences were identified between different Animal Health Divisions. Several factors summarise information about the number of cattle on the farm; these are highly correlated, and ultimately only FCattle is included in the draft multifactor model. The factors defining whether a group has recently been subject to a move or a change in diet are partially confounded: most observations are of groups which are positive for neither or both factors. However, the results overall are consistent with both factors having a protective effect. A new factor RecChange is therefore defined, describing whether or not a group has been subject to either change. The factors summarising the use of manure or slurry in silage production are highly confounded with the factor defining home silage production. More detailed analysis suggests that the spreading of slurry on pasture is the key factor in this group.

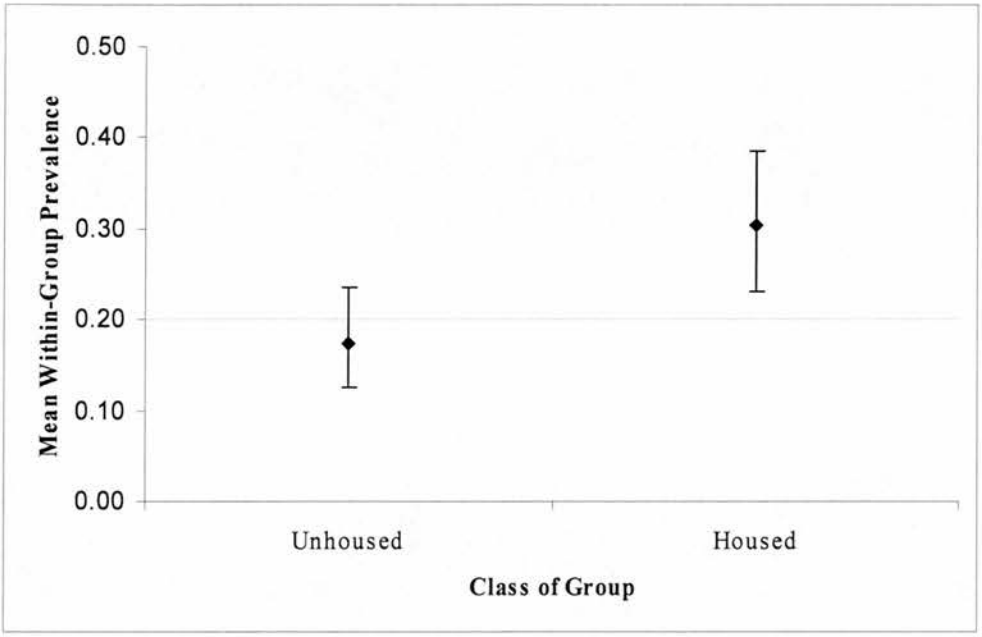
Using the stepwise procedures to evaluate candidate factors and variates, it is found that the factor defining whether a farm has sheep present is marginally statistically significantly protective. Hence, the factors Housed, FCattle, Housed.Source, Housed.RecChange, Silage_Slurry, Natural and Sheep are included in the Generalised Linear Mixed Model. Sampling Year, Sampling Month and Animal Health Division

are also included. Farm, County and Veterinary Practice were fitted as possible random effects, but only Farm proved statistically significant. Sheep and Housed.Source are found to have no statistically significant explanatory value and are removed from the analysis. The results of the final multi-factor model are summarised in Table 5.9.

Table 5.9 Results of the multifactor analysis: levels of shedding in positive groups.

Factor/Variable	Effect	Log Odds Ratio	se	*p*-value
Housed	Housed animals have higher mean prevalences.	1.319	0.33	<0.001
Categorised Number of Finishing Cattle (FCattle)	Farms with greater than 100 finishing cattle have significantly lower mean prevalences than those with less than 100.	-0.702	0.23	0.004
Housed/'Recent Changes in Housing or Diet' interactions	Farms with housed animals and changes during the previous 4 weeks have higher mean prevalences than farms with unhoused animals. This effect is not formally significant.	0.480	0.43	0.26
	Farms with housed animals and no changes during the previous 4 weeks have higher mean prevalences than farms with housed animals and recent changes.	0.891	0.33	0.007
Water sourced from natural supply	Farms with animals at pasture have lower mean prevalences if the water is from a natural source.	-0.708	0.35	0.04
Slurry spread on Farm	Farms with housed animals which spread slurry on their silage fields have a lower mean prevalence than farms with housed animals which do not. This result is not formally statistically significant.	-0.553	0.29	0.07
Animal Health Division	Scotland divided into three regions: Highlands; Central, Islands, North-East and South-East; and South West.			
	Highlands exhibits a significantly higher mean prevalence than the portmanteau region.	0.969	0.42	0.02
	The South West exhibits a significantly lower mean prevalence than the portmanteau region.	-0.600	0.28	0.03
Sampling Month	No significant effects identified. All variability explained by explanatory variables above, especially Housed.	Various	Various	0.23
Sampling Year	No significant effects identified.	Various	Various	0.61

Figure 5.4 Estimated means and 95% confidence intervals for the within-group fractional prevalence in housed and unhoused groups; values adjusted for other significant covariates and random effects in the multi-factor model.



No statistically significant management system effect was observed in the analysis of the basic data, and no further effects became apparent following the fitting of the multi-factor model. By contrast, the basic data showed evidence of a geographical bias in prevalence, and this trend remained in the multi-factor model, unexplained by any of the proposed explanatory factors. Hence it can be concluded that the farm level prevalences do vary with region, in a fashion which is not explained by the proposed explanatory factors.

In both analyses, examination of the Sampler and LabOperator factors confirm that there is no evidence of any effect due to operator bias at either the farm or laboratory.

Discussion

In a review of twenty-six published prevalence studies (Meyer-Broseta, *et al.*, 2001) the authors highlighted the problems caused by differing sampling and statistical methodologies. The microbiological methods used in this study were adopted as standard within the United Kingdom so the results may be compared with those obtained in England and Wales (Paiba, *et al.*, 2003) and subsequent studies, still to be published, carried out in Scotland under IPRAVE. When the present study was designed, the use of one gram faeces samples was considered to be more sensitive than rectal swabbing. The finding that VTEC O157 colonises lymphoid tissue at the recto-anal junction (Naylor, *et al.*, 2003) may explain why rectal sampling leads to higher prevalences at abattoirs (Chapman, *et al.*, 1997). With experimentally infected calves in the USA, enrichment culture of rectoanal mucosal swabs (RAMS) was found to be more sensitive than enrichment culture of 10g faeces samples, once colonisation was established (Rice, *et al.*, 2003). These workers however pointed out that in transiently infected animals, i.e. animals shedding for less than one week, only faecal culture detected the organism. The estimates of prevalence described in this paper have been shown to underestimate by approximately a factor of two the prevalence within faecal pats as the organisms are not evenly distributed within the pats (Pearce, *et al.*, 2004).

The estimation that 22.8% of groups of animals sampled contained at least one shedding animal fits closely with the findings of the longitudinal study in beef cows (Synge, *et al.*, 2003), when at least one shedding animal was found on 22% of 395 visits. That study clearly showed that repeat sampling increased the detection of VTEC O157, since after visiting each farm twelve times, the organism was detected

in 28 out of 32 (88%) herds. This variability is confirmed by the results in Table 5.3, where, based on relatively small samples, 2 farms (from a possible 7) exhibited statistically significant changes in prevalence on successive visits. The findings of these two studies, in consistently showing a higher than expected mean prevalence, led the Scottish Task Force on *E. coli* O157 to "advise farmers, other handlers and their families of the potential risks from contact with animals and their faeces" (Reilly, 2001). Clearly exposure alone does not always lead to human infection and illness.

In groups of cattle where shedding was detected, the level of observed shedding varied enormously (Figure 5.3). Although over forty percent of these groups provided only one positive sample, ten percent of the groups had all samples positive, suggesting that a high proportion of animals were shedding. Because the test has low sensitivity (Pearce, *et al.*, 2004) it is likely that in these herds at least some animals are shedding high numbers of organisms. This hypothesis is being considered by the IPRAVE project and the concept of super-shedders has been proposed (Matthews, *et al.*, 2006).

The proportion of VTEC isolates with genes encoding VT1 and VT2 was similar to the findings of the concurrent longitudinal study (Synge, *et al.*, 2003). The vast majority (59%) were phage type 21/28, showing a massive shift from the period 1992-95, when only 7% cattle isolates in Scotland were phage type 21/28. A similar shift occurred in human isolates during this period (Synge, 1998).

Although the main rationale for the analysis of the epidemiological dataset through two complementary analyses was to ensure the goodness of fit of the associated

statistical models, it is reasonable to consider that the explanatory factors identified in the absence/presence analysis may be those most associated with the introduction and continued survival of infection on a farm, while those identified in the shedding levels analysis will be those which change the level of contact of animals with bacteria on the farm or which affect the propensity of carrier animals to shed. Obviously, the latter effects will change the propensity for infection surviving within the local farm population, and it is interesting that the significant factors identified in the two analyses are so disparate. A good example of this is the effect of herd size. The analysis demonstrated a statistically significant increase in the likelihood of groups to be shedding if they were drawn from herds with more finishing cattle (Table 5.7). Interestingly, as reported in Table 5.9, farms with greater than 100 finishing cattle have statistically significantly lower mean proportions of animals shedding within the sample groups than farms with less than 100 finishing cattle.

There is no obvious epidemiological explanation of why the number of finishing cattle should have (apparently) opposing effects on the mean propensity to shed and the mean within-group prevalence. However, such an effect could arise from the interplay of the threshold properties of infection systems and the sampling scheme used in the prevalence study. It can be assumed that the between-animal infection rate of *E. coli* O157 within different farms is highly variable (perhaps explained by some of the risk factors listed in Table 5.9). The mathematical theory of epidemic systems (Anderson and May, 1991) would suggest that the probability of the infection dying out in small groups is higher than in larger groups. When a cross-sectional study is carried out, the sample of small groups will therefore tend to have proportionately more negatives. However, the samples from those small groups

which are positive will disproportionately be drawn from those farms with high transmission rates, and hence with higher mean within-group prevalences. Hence both observed effects could arise naturally from the infection dynamics of *E. coli* O157 in groups of different size.

In the univariate analysis it was found that farms that purchased cattle for finishing rather than breeding their own replacements were more likely to present shedding animals. The effect of sourcing is however confounded with farm size, since larger farms are more likely to have bought in animals, and when the farm size is included in the model, source ceases to have any formal statistical significance. Detailed analysis of the effect of group size on the risk of shedding on open and closed farms separately indicates that group size is the important factor in determining risk.

The finding that a higher maximum age of cattle in the sampling group is associated with a lower risk of the group being positive is consistent with earlier work. For example, in an USA study, 0.2% adults and 0.65% weaned calves were found to be shedding (Hancock, *et al.*, 1994). Groups sampled on dairy farms with beef cattle are at a higher risk of being positive than those from other farms. This could perhaps reflect management practices on this atypical category of farm. The suggestion that the presence of pigs on a farm is associated with a higher risk of shedding in cattle is intriguing. Pigs are not considered to be important in the epidemiology although clearly they can carry the organism (Borie, *et al.*, 1997, Chapman, 2000, Heuvelink, *et al.*, 1999). Contact between pigs and cattle on farms is, however, unlikely in most situations in Scotland, although indirect faecal contamination is probable.

The spreading of slurry on grazing land was shown to increase the risk of groups of housed animals shedding VTEC O157. The spreading of manure on pasture was protective for housed groups. This is possibly because the majority of farms spread either slurry or manure on the pasture and it is known that the composting effects of dung heaps reduce the levels of bacteria in the faeces. The longitudinal study (Synge, *et al.*, 2003) identified wild geese as a risk factor for shedding in grazing cattle, but no such effect was observed in the present study.

The proportion of groups of cattle found to be shedding decreased significantly from year one to year two and from year two to year three in the study (Figure 5.2). The observed differences may reflect high year to year variability rather than a trend. There were no differences between the mean proportions of animals shedding within positive groups in different years. The suggestion that prevalence is declining is being explored further in IPRAVE.

There was a cyclical effect with more herds shedding in the summer than the winter. This is in broad agreement with other studies. An early longitudinal study in a dairy herd in England showed two peaks of shedding, one in the early summer and one in November after housing (Mechie, *et al.*, 1997). A longitudinal study in cows (Synge, *et al.*, 2003) found the greatest number of groups shedding in the autumn months. Research in Aberdeen has demonstrated higher counts in the faeces of cattle in the summer months (Ogden, *et al.*, 2004). It is plausible that other, as yet unidentified, risk factors are influencing the group shedding risk in some months of the year. There was no variability in mean shedding proportions in positive groups between or within years that was not explained by other factors, predominantly housing.

The analysis showed that, while housed groups are no more likely to be shedding than grazing animals, the mean proportion of animals shedding in positive groups is statistically significantly greater in housed animals. This can perhaps be explained by the increased chance of bacterial transmission in housed animals or the greater chance of exposure from feed or water troughs (LeJeune, *et al.*, 2001). In the longitudinal study (Synge, *et al.*, 2003) groups of animals were more likely to be shedding when housed and an effect of bringing indoors was also noted. In the present study, housed animals, which had had changes in diet or management in the previous four weeks showed higher mean shedding proportions than unhoused animals, although this effect was not formally statistically significant. However, the mean proportion of shedding animals in housed groups which had not had such a recent change in diet or management was statistically significantly higher than in other housed groups. These results are consistent with a build up of exposure for housed animals from organisms colonising feed and water troughs.

Comparing groups of animals at pasture, cattle had lower mean shedding prevalences when they had access to a natural water supply. This may relate to the finding that water trough sediments in drinking troughs can be contaminated by cattle faeces and thence act as reservoirs for the spread of infection (LeJeune, *et al.*, 2001). Although cattle have occasionally been infected from natural watercourses, these results suggest that this is a low risk.

The study found no region of Scotland to be more or less likely to have shedding groups of cattle than any other, but within groups, the Highlands had significantly

higher mean shedding prevalences and the South West significantly lower mean shedding levels compared to the rest of Scotland. These geographical differences could not be explained by the other explanatory variables included in the multi-factor model.

In addition to the determination of the prevalence of VTEC O157 to a small tolerance, the large size and design of this study has facilitated an extensive analysis of the risk factors affecting the shedding of the organism. The two-stage analysis of the data has proved successful in identifying risk factors which may influence different aspects of the epidemiology. In particular, it is interesting to note that housing status, although very important in affecting the mean prevalence within positive groups, shows no evidence of having any influence on whether or not a group is positive. It is therefore likely to operate as a risk factor purely at the within-group level. The interpretation of this housing factor is facilitated by the observed effects of recent changes in housing or diet, allowing us to infer some aspects of the likely infection dynamic as animals are brought in from pasture from a cross-sectional study. Hypotheses about the likely transmission route of *E. coli* O157 will be informed by the findings about water supply and slurry and manure spreading, as well as the identification of pigs as a risk factor, while the effect of cattle age and group size can be explained by reference to experimental studies and mathematical biology respectively. In defining future research, the unexplained geographical and temporal variability might suggest alternative risk factors which vary in an unbalanced fashion across Scotland or across time. Perhaps most importantly of all, the apparent temporal decline in group prevalence, if continued into later years, would dramatically reduce the public health risk from *E. coli* O157 infection.

This study has produced a large volume of information, which is already being used by researchers in several disciplines within the IPRAVE project in an effort to further elucidate the epidemiology of VTEC O157 carriage in cattle.

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Chapter 6: PILOT PREVALENCE STUDY OF VTEC O157 IN SHEEP IN GREAT BRITAIN

Introduction

Verocytotoxin-producing *E. coli* (VTEC) were detected in faeces from sheep with diarrhoea as early as 1992 (Woodward, *et al.*, 1992). VTEC were detected in 66% of healthy sheep in Germany (Beutin, *et al.*, 1993). Naturally occurring VTEC O157 was detected in sheep in the USA (Kudva, *et al.*, 1996) and the UK (Chapman and Siddons, 1996). In a slaughterhouse study in Sheffield 22 out of 100 faecal swabs were positive for VTEC O157 (Chapman, *et al.*, 1997). In a small study at slaughterhouses in the Netherlands VTEC O157 was isolated from both ewes and lambs (Heuvelink, *et al.*, 1998).

In the investigation of the sources of outbreaks SAC associated lambing ewes with human illness (Allison, *et al.*, 1997). Milk was identified as a possible source of VTEC O157 (Rubini, *et al.*, 1999) and a pilot study of unpasteurized sheep and goats' milk offered for retail sale showed 50% ewe milk samples failed the standards prescribed by the Dairy Products (Hygiene) Regulations 1995 (Little and de Louvois, 1999). Sheep have been incriminated as a source of infection at an open farm (Chapman, *et al.*, 2000). VTEC O157 was isolated in high numbers from lamb sausages and burgers in Yorkshire (Chapman, *et al.*, 2000). As mentioned under the first section of this report an outbreak at a campsite in the Scottish Highlands was associated with the contamination of an untreated water supply by sheep (Licence, *et al.*, 2001). Also mentioned in Chapter 8 was an outbreak at a campsite associated with sheep faeces (Strachan, *et al.*, 2001). 100% of the faeces samples were positive for VTEC O157 and a count of $>10^6$ organisms per gram faeces was recorded.

Methods

Fifty sheep flocks were randomly selected from all regions of Great Britain. They were identified from a database for the Sheep and Goat Health Schemes. There are approximately 5000 flocks to select from, the maedi visna accreditation scheme members being mostly terminal sire breeders and the Premium Health Scheme being composed of commercial flocks. In this way the stratified sheep industry in Britain was represented. Adult sheep or hoggs (yearlings) were sampled ie they were all ruminants as opposed to pre-ruminants. In all cases fresh faeces samples were collected off the ground. The number of samples collected at each visit was determined using criteria that had been developed for a cattle prevalence study in Chapter 5. In summary, sample sizes were generated based on a model of within-herd prevalence assuming that 2% of herds would contain shedding animals. The shedding patterns on positive farms would be similar to those seen in data from farms previously investigated following human infection, varying around 10%. From this model the number of samples required to give an adequate probability of detecting that a herd contained cattle which were currently shedding was calculated. This power was set at 80% as a biologically acceptable value. For examples in a group of 20 ewes, 17 samples were collected, with 30 ewes 20 samples, 50 ewes 23 samples and 200 ewes 27 samples were collected. Samples were collected into sterile plastic containers and were tested within forty-eight hours of sampling.

A simple questionnaire was completed recording the name, address, county, number of sheep in the group, number of samples collected, date of sampling, sampler, breed of sheep, month of lambing, housed or outside, age-group i.e. ewes or hoggs and diet i.e. grass, hay, silage, turnips or concentrate feed.

All samples were examined by IMS as described under investigations above. The enumeration method is also described above.

Results

Fifty flocks were sampled, 25 from England and Wales and 25 from Scotland. The flocks were distributed in thirty-four counties. At least fifteen different breeds were included. All samples were collected between December and April. Twenty-two of the groups of sheep sampled were housed. Seven of the groups were yearlings while the remaining groups were adults. Between 11 & 27 samples were taken from each flock according to the group size.

One or more VTEC O157 positive samples were identified from four of the fifty flocks, a proportion of 0.080 with 95% confidence limits of 0.022 and 0.192. (This could be expressed as a **Group Level Prevalence of 8% with 95% confidence of 2% to 19%**) One positive sample was identified from each of three flocks but a fourth flock had eight positive samples.

Eleven out of 1117 samples were VTEC O157 positive, a proportion of 0.010 (i.e. an **Animal Level Prevalence of 1%**). An estimate of confidence limits for this proportion is not shown here for reasons given in the discussion.

Enumeration of VTEC O157 organisms in the groups with one positive sample, showed the samples to contain $<5x\ 10^2\ g^{-1}$, $<5x\ 10^2\ g^{-1}$ and $10,^5g^{-1}$ while in the group with multiple positives, counts ranged from $<5x\ 10^2\ g^{-1}$ to $>10^4g^{-1}$.

Three of the positive flocks were in Scotland (three different counties) and one in England. Two groups were housed and two outside. Two groups were being fed hay or silage and one was also being fed a concentrate ration. Three breeds of sheep were affected.

Discussion

This pilot study has suggested that the prevalence in sheep flocks is less than in cattle. In Scotland for example it was found that 8.5% of feeding cattle were shedding and 23% of herds had at least one shedding animal (Synge and Paiba 2000). The study has also demonstrated that, like in cattle herds, flocks may occasionally have multiple shedding animals.

Diet and management may influence the shedding of VTEC O157 by sheep. For example groups of lambs, that were experimentally infected, shed sooner and for longer when confined and fed pellets of alfalfa. All the animals when released onto rangeland shed organisms at the same rate and for approximately 15 days (Kudva, *et al.*, 1995). The same workers also showed that a brief period of starvation could induce shedding and then subsequent clearance of the organism. In another study they found that feeding hay (high in fibre but low in energy and protein decreased shedding in experimentally infected sheep compared to sheep fed alfalfa and corn but no hay (Kudva, *et al.*, 1997). In a limited study the same authors suggested that the season of the year could have an important effect on presence or absence of shedding (Kudva, *et al.*, 1996, Kudva, *et al.*, 1997). In a Sheffield study 17 out of the 22 isolates were made during the summer (Chapman, *et al.*, 1997). In a recently published slaughterhouse study there was evidence of higher faecal shedding by sheep in the summer (Paiba, *et al.*, 2002).

There is some evidence from our study so far compared to investigations following outbreaks and from the published literature that there may be seasonal variation. It is hypothesised that this may relate to the feeding of concentrates which takes place around lambing and during early lactation. In this study all samples were collected between December and April. Sampling for a complete year would have been

preferable. A personal communication from Ian Ogden, University of Aberdeen, has shown large numbers of high shedding sheep in Grampian in the summer of 2003. His study may not be comparable but it is still recommended that our study is carried on through the summer months. If there is a large increase in shedding it may be possible to associate this with season, lambing or feeding. Increasing the number of flocks sampled over a year would make the establishment of associations more likely.

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Chapter 7: PILOT PREVALENCE STUDY OF VTEC O157 IN DEER IN SCOTLAND

Introduction

In the USA white-tailed wild deer sharing rangeland with domestic cattle were shown to shed VTEC O157 (Rice, *et al.*, 1995) and the organism has been isolated from wild deer in Japan (Asakura, *et al.*, 1998), while farmed deer in the UK were also identified as a potential source of human infection (Chapman and Ackroyd, 1997). In a small prevalence study five out of 212 (2.4%) white-tailed deer were shedding VTEC O157 in pastures in North Kansas (Sargeant, *et al.*, 1999). A recent report looking at a number of wildlife species in USA found five out of 630 white-tailed deer shedding, using enrichment but not IMS (Rice, *et al.*, 2003). Low prevalences were found in deer sharing pasture with cattle that were shedding higher numbers (Fischer, *et al.*, 2001). VTEC O157 was not detected in Reindeer in Norway (Wasteson, *et al.*, 1999) nor in Finland (Lahti, *et al.*, 2001), where the prevalence of faecal shedding was estimated at <0.22% with 95% confidence interval.

Six young white-tailed deer were experimentally infected with VTEC O157. Faecal shedding was observed from day one, but continued to shed decreasing numbers for the 26 days of the trial (Fischer, *et al.*, 2001). This is comparable to studies in cattle and sheep.

VTEC have been detected in venison (Thoms, 1999). Phylogenetic diversity as described by some workers suggests similar strains of VTEC in deer and sheep, which are sometimes dissimilar to human isolates (Asakura, *et al.*, 2001). The case described in the first part of this report however clearly showed how deer can be the source of

human infection. An outbreak of human disease was associated with the consumption of venison jerky a marinated and dried meat (Keene, *et al.*, 1997). Another outbreak of VTEC O157 was associated with the drinking of a particular brand of unpasteurised apple juice (Cody, *et al.*, 1999). Deer were frequent visitors to the orchard.

Methods

Locating deer sites and sampling methods proved difficult. We were twice let down by organisations that initially said they could help. The sites sampled initially were visited and faeces collected off the ground. It was then established with Forest Enterprises that rangers would collect samples direct from carcasses, as they were gralloched (removal of thoracic and abdominal organs) after shooting.

The numbers of samples collected at each site were determined from the model described in the sheep study.

The microbiological methods are described above.

Results

Five sites were sampled to 23 January 2003. Two sites were farmed Red deer, two were wild Red deer and one site was wild Roe deer. Seventy-three samples have been tested. No sample was positive for verocytotoxin producing *E. coli* O157. A further 711 faeces samples from 45 sites were submitted from shot deer. All these samples were also negative for VTEC O157. The numbers of each species of deer sampled were 427 Roe, 270 Red, 84 Sika, 3 Fallow and one unlabelled.

Discussion

This study suggests that the prevalence of VTEC O157 in deer in Scotland is low. Since the study was carried out the site of colonisation of VTEC O157 in the bovine has been described (Gally, *et al.*, 2003, Naylor, *et al.*, 2003). It is not known whether there is a similar site in deer, but if this was the case sampling from the rectum rather than faeces might have an effect on isolation rates. The incident (no. 54) described in Chapter 8 of this theis, suggests that the presence of high shedders in deer may be of significance in giving rise to human infection. While there is little evidence of seasonal shedding in the literature, it is possible that there may be an effect. It is therefore preferable that this study also is extended into the spring summer and autumn.

Acknowledgements

This Chapter is based upon a section written by the author in a Food Standards Agency Report (Stanfield, *et al.*, 2004). The project was funded by the Food Standards Agency.

Chapter 8: ANIMAL SOURCES OF VTEC O157 INFECTION FOR HUMANS IN SCOTLAND – 1993 to 2003

Introduction

Soon after verocytotoxin-producing *Escherichia coli* (VTEC) O157 were associated with disease in man, cattle were implicated as carriers (Martin, *et al.*, 1986). The organism was first isolated from cattle in Scotland six years later (Synge and Hopkins, 1992). Since then SAC have been involved in following up incidents of human infection.

Methods

Farms were visited and a history was taken in an attempt to determine the possible routes of infection between animals and the infected human(s). Any species of animal thought likely to be a source was sampled. In most cases the number of samples collected was sufficient to be 90% confident of isolating the organism in a population with 5% prevalence of infection. Incidents investigated were either single human cases or outbreaks. In the investigation of some incidents multiple farms were sampled and on occasion repeat sampling of a farm or other site was carried out.

In the first investigation direct culture on sorbitol MacConkey agar was employed, but since then the more sensitive immunomagnetic separation (IMS) technique has been employed. Initially the method developed in Sheffield was employed (Chapman, *et al.*, 1994), but since 1996 the antibiotics in the enrichment medium have been omitted since this was found to improve the sensitivity of the test (Synge, 1998). Briefly 1g samples of animal faeces were suspended in 20 ml of buffered peptone water (BPW)

(Oxoid, Basingstoke, UK). Two mud samples from around a water tank were treated similarly. All were incubated at 37°C for 6 h, after which 1 ml from each pre-enrichment broth was removed and mixed with 20 µl of *E. coli* O157 specific-antibody-coated beads (Dynal, Oslo, Norway) on a rotary mixer for 30 min. The sample vials were then loaded on to an immunomagnetic separator (Dynal) and left for 3 min to allow the beads and any bound *E. coli* O157 to become attached to the side of the vial. The broth was then removed and replaced with 1 ml of 10 mM phosphate buffered saline (pH 7.4) with 0.05 % Tween 20 (PBST) (Sigma, Diagnostics, Poole, UK). The tubes were removed from the rack and shaken then placed back on the magnet for a further 3 min and the PBST removed. Two further washes in PBST were performed after which the beads were resuspended in 50 µl of PBST and inoculated on to Sorbitol MacConkey agar (Oxoid) plates with cefixime and tellurite added (Mast Diagnostics, Bootle, UK) (CT-SMAC). Plates were incubated at 37°C for 18-24 h and any pale non-sorbitol fermenting colonies (NSF) which agglutinated with *E. coli* O157 latex reagent (Oxoid) were sent to the Scottish *E. coli* Reference Laboratory for typing.

Colonies that did not ferment sorbitol were tested by slide agglutination with O157 antisera. Positive isolates were forwarded to the Scottish *E. coli* reference laboratory for confirmation and typing. Multiplex PCR for genes encoding the verocytotoxins, phage typing and pulsed field gel electrophoresis were performed on most isolates. In some cases plasmid profiling was also carried out.

In the most recent outbreaks positive samples were further tested to enumerate the *E. coli* by preparing a 1 in 10 dilution of faeces in Maximum Recovery Diluent and plating two 0.5 ml volumes on to each of two CT-SMAC plates and 0.1 ml on to a

CT-SMAC plate (i.e a 1/10 and 1/100 dilution of faeces). The total number of *E. coli* O157 on the two plates was counted and the number per g of faeces calculated.

Results

The numbers of farms investigated in each year and numbers of farms positive for VTEC O157 is shown in Table 8.1. Records show that out of 91 farms or sites sampled, 31 were positive for VTEC O157.

Table 8.1 Farm Investigations by SAC by Year

Year	No. Farms investigated	No. VTEC O157 positive
1993	2	2
1994	34	8
1995	13	3
1996	14	5
1997	10	5
1998	4	0
1999	4	1
2000	2	2
2001	4	1
2002	2	2
2003	2	2
Total	91	31

Table. 8.2 summarises the records available from 57 incidents investigated between 1993 and 2003. In the first twenty-five incidents investigated all animal isolates were from cattle although other species were sampled on occasion. Subsequently as shown there were also isolations from goats, sheep, horses, a goose, deer and a cat. In some incidents multiple farms were investigated, not all farms were positive eg in incident 9, 18 herds were sampled and only one was positive. Sometimes the VTEC O157 isolated did not match (ie it was distinguishable from) the human isolate eg incident 2. In incident 4 the isolate was close but distinguishable, while in incident 10 distinguishable and indistinguishable isolates were obtained.

As shown in Table 8.2, according to our records, in at least 17 out of 57 incidents an isolate was recovered from an animal indistinguishable from the human isolate. Features of the isolates such as the verocytotoxins produced, the phage type and the fingerprint of the genome by pulse field (and the plasmid profile when determined) corresponded in all these cases. In five herds VTEC O157 of a different type was isolated and in a further 30 herds no VTEC O157 was isolated. In some incidents the records as to whether isolates matched are not complete. In the incidents where a matching isolate was found, phage type 2 was encountered six times, while phage types 49, 54 and 28 were each seen once only. Phage type 21/28 initially denoted as pt28 first appeared in 1994, but in the last three years this was the only phage type encountered.

Table. 8.2 Isolations of VTEC O157 from animals in fifty-seven incidents investigated and comparison with human isolates

Year	Incident	Health board	Phage type	VT type	Management system	No. samples	No. positive	Match
1993	1	Orkney	49	2	Beef suckler	84	1	Yes
1993	2	Grampian	2	2	Dairy	40	1	No
1994	3	Ayrshire & Arran	54	1+2	Dairy	69	7	Yes
1994	4	Grampian	32	2	Beef suckler	50	7	? No
1994	5	Grampian	2	2	Feedlot	45	0	-
1994	6	Borders	2	2	Housed young stock	43	3	Yes
1994	7	Shetland	1	1+2	Croft	31	0	-
1994	8	Lothian	49	2	Feedlot, sheep & horse	52	0	-
1994	9	Lothian	2	2	18 dairy herds	359	13	Yes
1994	10	Dumfries & Galloway	2	2	3 dairy herds	66	5	Yes & No
1994	11	Tayside			Small-holding	8	0	-
1994	12	Ayrshire & Arran			Pony trekking & open farm	32	0	-
1994	13	Borders	2	2	Beef suckler	22	7	Yes
1994	14	Tayside	2	2	Dairy	14	1	Yes
1994	15	Grampian	28	2	Calf rearer	13	7	Yes
1994	16	Grampian	28	2	2 dairy herds	21	0	-
1995	17	Tayside			Dairy	46	0	-
1995	18	Grampian	2	2	Dairy	51	3	No
1995	19	Fife			Dairy	46	0	-
1995	20	Dumfries & Galloway	2	2	Beef suckler	62	5	?
1995	21	Highland			Grazing cattle	20	0	-
1995	22	Grampian	49 & 2	2	Beef suckler	14	5	?
1995	23	Grampian			Cattle	1	0	-
1995	24	Grampian			Beef & dairy	11	0	-
1995	25	Grampian			Cattle	11	0	-
1996	26	Fife	28	2	Goats	8	4	Yes
1996	27	Shetland	2 then 28	2	Goats, pony & cattle	11	7	Yes
1996	28	Grampian			Goose	2	1	?

Year	Incident	Health board	Phage type	VT type	Management system	No. samples	No. positive	Match
1996	29	Grampian			Sheep & Cattle	6	2	?
1996	30	Grampian			Sheep	19	14	?
1996	31	Grampian			Pigs	15	0	-
1997	32	Grampian			Cattle	8	0	-
1997	33	Grampian			Ostriches & horses	10	0	-
1997	34	Grampian			Sheep	8	0	-
1997	35	Grampian			Horses	3	0	-
1997	36	Grampian	21/28	2	Cattle	15	6	?
1997	37	Grampian	21/28	2	Cattle	44	11	?
1997	38	Grampian			2 horses & 1 goat	3	0	-
1997	39	Grampian	21/28	2	Cattle	19	6	?
1997	40	Grampian	21/28 2	2 2	Cattle Horse goats	6 1 11	5 1 0	?
1997	41	Grampian	21/28	2	Cattle Horses	13 3	6 0	
1998	42	Shetland	21/28	2	Cattle	8	4	?
1998	43	Grampian			Cattle Goats pony & dung heap	20 5	0 0	-
1998	44	Grampian	21/28	2	Cattle	10	0	-
1999	45	Highland			Sheep (water)	23	2	Yes
1999	46	Grampian	21/28	2	Goat, ewes, lambs, goose & ducks	27	17	Yes
2000	47	Orkney	21/28	2	Cattle	43	3	?
2000	48	Grampian			Sheep (camping)	28	28	Yes
2001	49	Ayrshire & Arran	21/28	2	Cattle (camping)	14	1	Yes
2001	50	Shetland			Pet lamb	1	0	-
2001	51	Shetland			Geese	2	0	-
2001	52	Forth Valley			Dog & Rat	2	0	-
2002	53	Highland	21/28	2	Cattle (water)	25	3	Yes
2002	54	Highland	21/28	2	Deer (water)	15	4	Yes
2002	55	Forth Valley			Rhea, goats, pigs, llamas & geese	21	0	-
2003	56	Ayrshire	21/28	2	Horses	10	2	Yes
2003	57	Highland	Sorb ferm.		Cattle & cat	29	5	(Yes)

Direct contact

The first link established between cattle and man was in Orkney (Synge, *et al.*, 1993) VTEC O157 had been isolated from a child with diarrhoea and the medical investigation noted that there was a farm adjacent to the child's house. The child had visited the farm and the family pet dogs roamed freely through the cattle sheds and dung heap. On visiting the farm eighty four samples were collected and all were screened for VTEC O157. One sample from a dung pat yielded the organism. Typing of the organism showed it to be indistinguishable from the human isolate. Both isolates were VT1 negative and VT2 positive, both were phage type 49, both had the unusual plasmid profile described as 90b:4 and the profiles of the isolates were indistinguishable by pulse field gel electrophoresis.

Incident no. 6 involved a suckler herd in Borders Region, where an eight month old child was regularly exposed to a cattle environment when he was taken daily in a pushchair to the cow shed where his parents worked. Following the onset of bloody diarrhoea the child developed haemolytic uraemic syndrome.

VTEC O157 had been isolated two years previously from the herd associated with incident no.14, but on that occasion a different phage type had been identified. The father of the affected child was a dairyman and took the child to the farm.

Incident no. 15 was associated with a five year old child petting young calves.

Incident nos. 36, 37 & 39 all involved the grandchildren of a farmer in each case. In incident no. 37 other family members were also affected.

Raw milk

Incident no. 3 was a dairy herd in Ayrshire at which the 53 year old farmer's wife regularly fed calves and drank raw milk. She developed severe bloody diarrhoea requiring admission to hospital.

Pasteurised milk

Incident no. 9 was an extensive epidemiological investigation following a major community outbreak in Lothian associated with a milk processing plant (Upton and Coia, 1994). Cattle on eighteen dairy farms (herd nos. 9 to 26) supplying the plant were investigated. In only one of these (herd no. 26) was VTEC O157 isolated. The organism was recovered from 16 of the 35 faecal samples examined and was indistinguishable from the human isolates.

Contaminated environment

The complexity of the situations that can be encountered was demonstrated by incident no. 10. Following bloody diarrhoea in a 15 month old child in Dumfriesshire who had been admitted to hospital, three herds adjacent to the child's home (27-29, two dairy and one suckler) were investigated. In two herds (27 and 28) VTEC O157 of matching phage and verocytotoxin type to the human case was isolated. In one of these herds (28) and a third herd (29) VTEC O157 of a different type was also isolated. There was considerable regular contact with cattle faeces in the environment surrounding the house as the cattle frequently passed along the road.

A six year-old child developed bloody diarrhoea (incident no. 42) associated with cattle grazing around the house.

Possible water spread to cattle and then direct contact

Incident no.13 involved children from two families. Dairy cattle grazed over the fence from the first family identified, but the source of infection was traced back to a beef suckler herd where the second family lived and the uncle of the other children worked. The organism was isolated from grazing cows and calves. This farm was downstream of the farm associated with incident no. 6 and the phage type and verocytotoxin type were the same.

Hard Cheese

Incident no. 16 was a large outbreak associated with hard cheese. VTEC O157 was isolated from a hard cheese on two occasions that was epidemiologically linked to the human outbreak. The organism was not isolated from the milk used to make the cheese or from the cows that produced the milk, but it was isolated from the cheese maker.

In a smaller outbreak involving farm cheese (incident no. 44) again the organism was isolated from the cheese but not the cattle producing the milk.

Dogs as possible vectors

Incident nos. 20 & 22 require further clarification to determine linkage. A dog was suspected as a vector from the cattle in incident no. 22. The dog was the likely way that infection was carried from a herd of cows to a young girl. Similarly in incident no.1 dogs could have brought the infection from the farm to a young boy or vice versa.

Cat as possible vector

In incident no. 57, which involved a sorbitol fermenting strain of VTEC O157, a cat was positive as well as the cattle. The cat frequently sat on the affected person's lap, while she had no contact with the cattle.

Lambing ewes

Amongst incidents investigated are some that have been documented. For example the association between lambing ewes and human infection (Allison, *et al.*, 1997).

Horses

In incident no. 56, a mare and foal were associated with a human case. In another incident (no. 27) goats, cattle and a pony all were shedding the organism, while in incident no. 40 cattle and a horse were shedding the organism.

Contaminated camp sites

A scout campsite was contaminated by sheep droppings and under wet conditions scouts became infected (incident no. 48)(Howie, *et al.*, 2003). Similarly a Girl Guide campsite was contaminated by cattle faeces (incident no. 49). In both cases enumeration suggested that high shedders were involved. In the case of the cattle a sample from one animal had <3 organisms per gram of faeces, while another had 2 x 10^3 organisms per gram. The counts in the sheep associated outbreak showed one lamb shedding $>10^6$ and two ewes shedding $10^4 - 10^5$ organisms per gram faeces (Strachan, *et al.*, 2001).

Goats milk

There was an incident (no.26) involving the death of a child associated with contact with goats. In another incident VTEC O157 was isolated from cattle and goats on the same holding (no. 27).

Goats cheese

In response to clinical disease in four primary school children (incident no. 46), the whole class of thirty children and four adults, who had also consumed some home-made goat's cheese were screened for VTEC O157. Twenty-four children and three adults tested positive. An indistinguishable organism was isolated from the faeces of the goat that produced the milk to make the cheese as well as ewes, lambs, geese and ducks on the same holding.

Sewage

In an incident in Orkney (no. 34) it was concluded that a man was infected by lying in a drainage ditch, while scaring wild geese, from the outflow of a septic tank. It was thought that he may have subsequently infected his cattle.

Untreated private water supplies

a) sheep

Incident no. 44 was an outbreak of illness at a campsite, associated with verocytotoxin-producing *Escherichia coli* (VTEC) O157, which was linked to an untreated water supply (Licence, *et al.*, 2001). The water supply in that case had been thought to be a spring but in fact was surface water from a hillside grazed by sheep. An indistinguishable VTEC O157 was isolated from the human cases, the water and from some sheep faeces collected on the hillside (Figure 8.1).

b) cattle

In the second incident (no. 53), where people at a campsite were affected, the water source was a spring. Cattle grazed around the collection tank and just prior to the outbreak there was heavy rain. Examination of the tank showed that the water level outside the tank had been higher than the overflow pipe and thus surface water from the surrounding area could easily have flowed into the tank (Figure 8.2).

VTEC O157 indistinguishable from the human isolates was isolated from the water, some cattle faeces adjacent to the tank and three out of 25 samples of faecal pats collected from 50 cattle that had been moved away from the tank two weeks previously. Less than 50 organisms per gram of faeces were present in each of the positive samples. The organism was also isolated from five litre samples of water that were filtered and the membranes enriched in buffered peptone water before immunomagnetic separation (IMS).

c) deer

The third water associated incident (no. 54) involved only a single human case but the history indicated that the patient had stayed in a house on a remote Highland estate. In this situation the water supply was taken from a burn running down a hillside

Figure 8.1. Catchment area for water supplying campsite from which sheep and deer faeces were collected.

Figure 8.2. Collection tank for spring. Heavy rainfall, with inadequate drainage, had caused surface water from the surrounding area grazed by cattle to flow into the tank.

Figure 8.3. Dam for collection of private water supply. Infected deer faeces were collected higher up alongside the burn.

(Figure 8.3). VTEC O157 was isolated from the water supply. Following this fifteen samples of red deer faecal pellets were collected from the ground alongside the burn and processed as above. Of these four yielded VTEC O157. Three of these had less than 50 organisms/g, but one sample had 7.5×10^4 organisms/g.

The isolates from the human patients, the water and the animals were indistinguishable by VT type, phage type and pulsed field electrophoresis in each incident.

The possible methods of spread of infection, where animals were implicated are summarised in Table 8.3.

Table 8.3 Incidents Related to Possible Sources of Infection

No. of Incidents	Route	Source
1	Improperly pasteurised milk	Dairy cows
3	Raw milk on direct contact	Cattle, Goats
7	Direct contact or contamination of food by persons handling the cattle	Cattle
2	Contaminated environment	Cattle
2	Camping Field	Sheep, Cattle
3	Untreated private water supply	Sheep, Cattle, Deer
3	Companion Animals	Dog, Cat, Horses
3	Handling sheep (lambing)	Sheep
1	Cheese	Goat

Discussion

This report gives an insight into the microbiologically confirmed routes of infection from animals to man of VTEC O157. SCIEH have provided information on outbreaks. Many of the incidents investigated by SAC are sporadic cases. Case control studies have suggested the importance of animal excreta for sporadic human cases (Locking, *et al.*, 2001). Direct contact has been confirmed to be a very important source of infection. Cattle are the most common source encountered but

examples of sheep, goats and horses were confirmed. There was circumstantial evidence of dogs or cats possibly acting as vectors from cattle.

Water-borne outbreaks were reviewed (Chalmers, *et al.*, 2000). Never previously have deer been incriminated as the source of contamination of a water supply. An outbreak involving the contamination of a public supply in Walkerton, Canada has been described (McQuigge, *et al.*, 2000). VTEC O157 have been isolated from deer in the USA (Rice, *et al.*, 1995) and the UK (Chapman and Ackroyd, 1997) but they have not previously been linked to water supplies.

This report highlights the importance of contaminated environments and sporadic cases are described when children are exposed around their homes. The two examples of contaminated campsites have already received attention.

Very frequently the cases affected were the families of farmers or farm workers. Clearly this sector of the population, far from being immune as is popularly believed, are particularly at risk.

Food other than cheese has not been incriminated in this study. This is in contrast to a study in England & Wales (Paiba personal communication), where 17 out of 48 incidents investigated food was the probable vehicle. The small proportion of food related incidents in Scotland may relate to better practices in meat plants, butchers shops and kitchens. Another factor however is that it is difficult to identify the source animals in food-borne outbreaks (Stanfield, *et al.*, 2004).

The Higher proportion of the Scottish population in contact with farm animals and their faeces compared to England & Wales could easily explain why VETEC O157 is more prevalent per unit population in Scotland. It is apparent from this study that most of the incidents were in rural areas where there is a high cattle population. The map (Figure 8.4) shows the cattle dense areas of Scotland.

Acknowledgements

This Chapter is based upon a section written by the author in a Food Standards Agency Report (Stanfield, *et al.*, 2004). The compilation of this report was funded by the Food Standards Agency. The follow up studies of human incidents were initially done as research, but continued under the Scottish Executive Environment and Rural Affairs Department funded surveillance of zoonotic diseases. Staff from all eight SAC Disease Surveillance Centres were involved with this work. Particular thanks is extended to D. Gray of SAC in Aberdeen for invaluable help in collating the data. Investigations were carried out at the behest of Consultants in Public Health Medicine (CPHMs) and the Scottish Centre for Infection and Environmental Health (SCIEH) acted as facilitators.

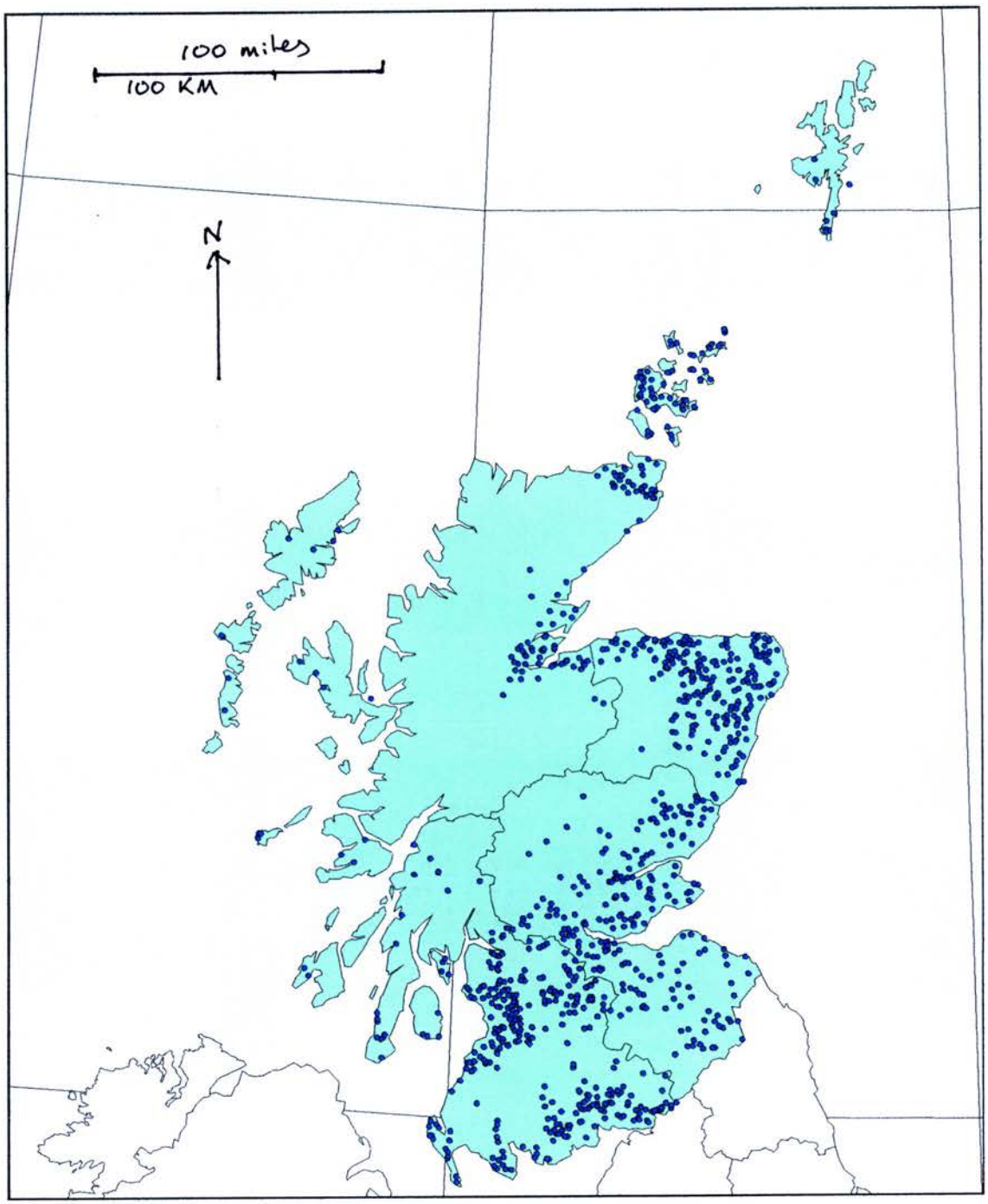


Figure 8.4 Cattle dense areas of Scotland

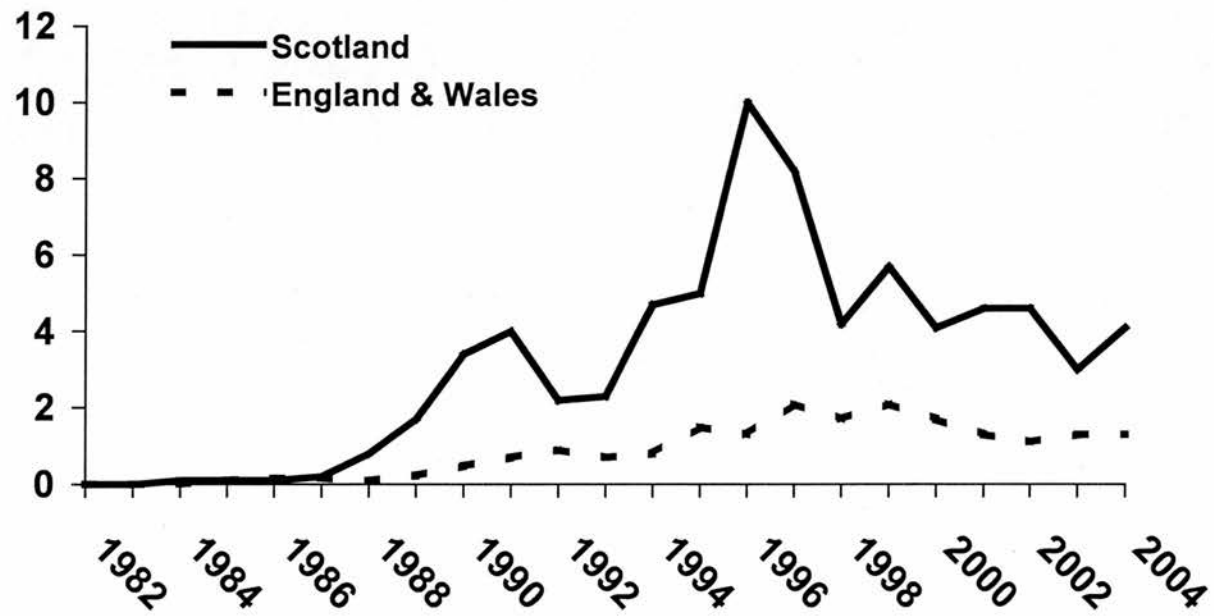
The dots represent individual farms sampled in the prevalence study (Chapter 5).

Chapter 9: GENERAL DISCUSSION, CONCLUSIONS AND FURTHER WORK

Regional differences in Prevalence

A recent report from Health Protection Scotland (formerly SCIEH) reviewed the enhanced surveillance and reference laboratory data for VTEC in Scotland during 2003 and gave some information for 2004 (Locking, *et al.*, 2004). The rate per 100,000 population decreased to 3.0 in 2003 but rose again to 4.1 in 2004.

Figure 9.1 Reported cases of VTEC O157 per 100,000 population in Scotland and in England & Wales (data from Health Protection Scotland and Health Protection Agency)



As shown in Figure 9.1 despite the rise in 2004 there is an apparent slow decline in the number of reported cases year by year in the last decade. It may be that the decline

is related to measures taken and in particular following the recommendations of the Task Force on *E. coli* O157 (Reilly, 2001). Other factors such as rainfall probably influence the numbers of cases especially when infection comes from untreated water supplies. A significant reduction in the number of groups of beef finishing cattle that were shedding VTEC O157 was recorded between the years 1998, 1999 and 2000 (chapter 5). Foot and mouth disease prevented any measurement in 2001 but the prevalence was recorded in 2002 and 2003 by identical methodology as part of the IPRAVE project. Preliminary results suggest that the trend is continuing (personal communication M Pearce), but full analysis is eagerly awaited. A downward trend in the number of cattle shedding would be expected to affect the human case rates.

Enhanced surveillance of VTEC O157 in Scotland has shown the majority of human cases to be sporadic. For example in 2003, 27(17.6%) cases were related to general outbreaks and the remaining 126(82.4%) cases were apparently sporadic (Locking, *et al.*, 2004). General outbreaks are defined as incidents involving members of more than one household. The same paper reported that 86.7% of symptomatic cases were considered to be primary with only13.3% being attributed to person to person contact.

A case control study has shown contact with farm animal faeces to be a risk factor (Locking, *et al.*, 2001). While it is acknowledged that some cases arise from foreign travel, food sources and person to person contact, it is hypothesised that the majority of sporadic cases arise due to contact with farm animal faeces. Infection is most often related to direct contact with animals, environmental contamination or through untreated water supplies. From the studies described in this thesis, it is clear that cattle

faeces carries a high risk of being infected, while faeces from sheep, deer and other animals pose a lesser risk.

The case rate per 100,000 population in Scotland remains consistently approximately three times that in England and Wales. There are also regional variations both in Scotland and in England & Wales. Data for the last five years extracted from the FSA Risk Assessment Project (Stanfield, *et al.*, 2004) and the recent Health Protection Scotland Report (Locking, *et al.*, 2004) is shown in Table 9.1.

Table 9.1 NHS Board of residence in Scotland: *E. coli* O157 enhanced surveillance 1999–2003(n=1100) (data from Health Protection Scotland)

	1999		2000		2001		2002		2003		Total	
NHS board	No	Rate	No	Rate	No	Rate	No	Rate	No	Rate	No	Average Annual Rate
Ayrshire & Arran	5	1.3	13	3.5	30	8.1	25	6.8	16	4.4	89	4.8
Argyll & Clyde	7	1.7	10	2.4	11	2.6	20	4.8	11	2.6	59	2.8
Borders	8	7.6	3	2.8	7	6.5	6	5.6	4	3.7	28	5.2
Dumfries & Galloway	27	18.2	12	8.1	12	8.1	8	5.4	8	5.4	67	9
Fife	22	6.3	15	4.3	9	2.6	5	1.4	10	2.8	61	3.5
Forth Valley	10	3.6	13	4.7	11	3.9	17	6.1	11	3.9	62	4.4
Greater Glasgow	16	1.8	12	1.4	22	2.5	14	1.6	9	1	73	1.7
Grampian	99	18.7	70	13.3	62	11.8	71	13.6	30	5.7	332	12.6
Highland	6	2.9	2	1	9	4.3	2	1	3	1.4	22	2.1
Lanarkshire	18	3.2	11	2	21	3.8	17	3.1	18	3.3	85	3.1
Lothian	24	3.1	20	2.6	23	3	28	3.6	15	1.9	110	2.8
Orkney	9	46.3	3	15.6	2	10.4	4	20.8	1	5.2	19	19.7
Shetland	8	35.5	3	13.5	4	18.2	0	0	2	9.1	17	15.3
Tayside	22	5.6	14	3.6	20	5.1	14	3.6	15	3.9	85	4.4
Western Isles	1	3.7	0	0	0	0	0	0	0	0	1	0.7
All Scotland	282	5.6	201	4	243	4.8	231	4.6	153	3	1110	4.4

The map shown in Figure 9.2 shows the rate of incidents per 100,000 population expressed as five year means between 1999 & 2003. Because the populations of some

health board areas are very low, eg Shetland, Orkney and Western Isles are each less than 30,000, the significance of regional variation year by year is unlikely in these areas. Focusing on health board areas with greater than 500,000 population, i.e. Greater Glasgow, Grampian, Lanarkshire and Lothian, the regional variation in VTEC O157 incidence is maintained from year to year as illustrated in Figure 9.3. Grampian consistently has the highest rate and Greater Glasgow the lowest.

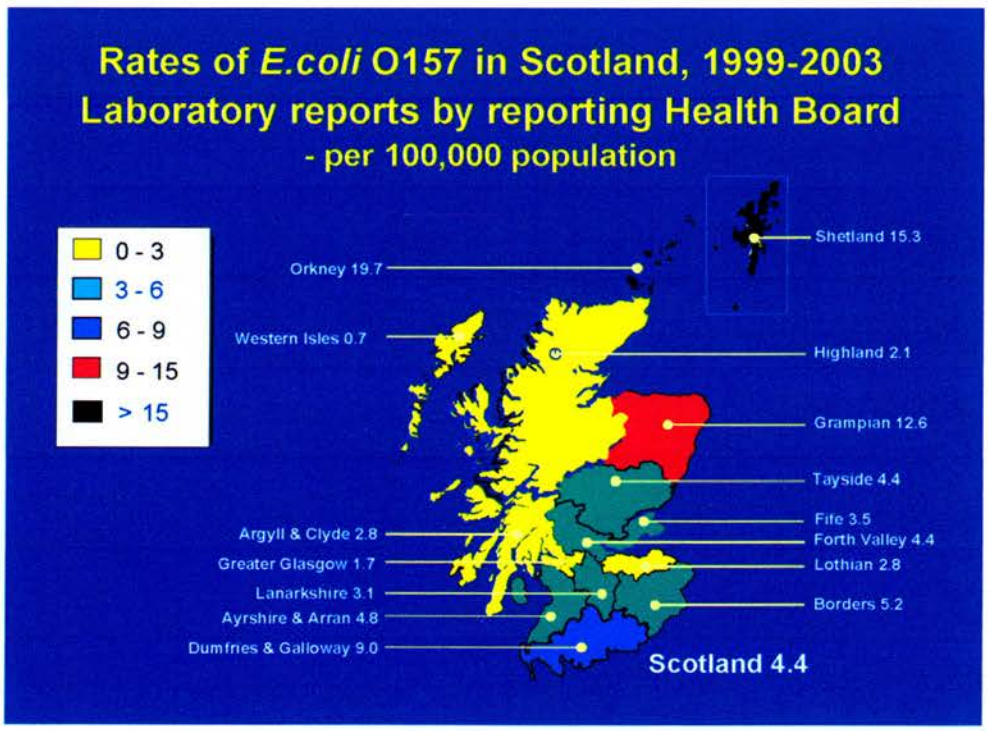


Figure 9.2 Human incidence of VTEC O157 in Scotland by Health Board - five year mean values. (Health Protection Scotland data)

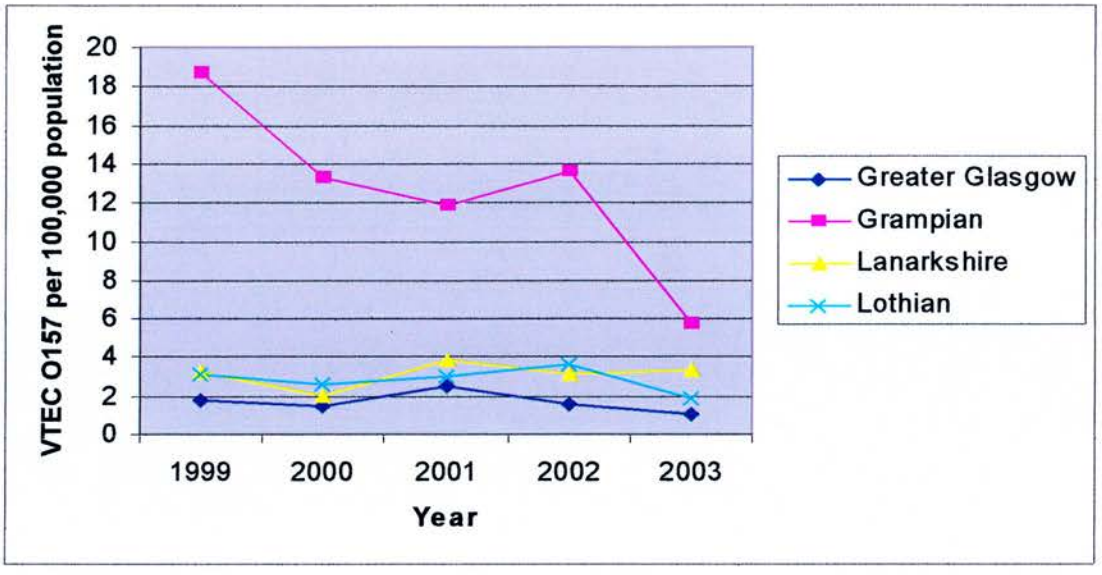


Figure 9.3 Human incidence of VTEC O157 in Scotland year by year over the five year period 1999 and 2003 in the four Health Board areas with greater than 500,000 population

Data for England & Wales provided by the Health Protection Agency for the FSA Risk Assessment Project (Stanfield, *et al.*, 2004) for a six year period is shown in Table 9.2. Up-to-date figures provided by the Health Protection Agency including data which shows the rates being higher in summer than winter, when more people are out of doors, are shown in Table 9.3.

Table 9.2 Geographical distribution of Verocytotoxin-producing *E. coli* O157 in England and Wales: 1995 to 2000 (n = 5409)

	1995		1996		1997		1998		1999		2000		Total	Average
Region	No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate
Northern & Yorkshire	126	1.9	109	1.64	150	2.26	162	2.45	244	3.66	234	3.51	1025	2.57
Trent	65	1.36	105	2.19	156	3.25	78	1.63	97	2.02	93	1.94	594	2.07
Anglia & Oxford	63	2.15	62	1.07	113	2.13	82	1.55	75	1.41	70	1.32	465	1.61
North Thames	43	0.63	50	0.73	94	1.37	89	1.3	69	1	66	0.96	411	1
South Thames	55	0.81	46	0.68	71	1.05	87	1.29	112	1.65	80	1.18	451	1.11
South & West	165	2.51	110	1.68	162	2.47	138	2.1	147	2.24	119	1.81	841	2.14
West Midlands	106	2	57	1.07	137	2.58	76	1.43	85	1.6	68	1.28	529	1.66
North West	88	1.33	85	1.29	161	2.43	115	1.74	189	2.86	120	1.81	758	1.91
Wales	81	2.78	36	1.23	43	1.47	63	2.15	66	2.26	46	1.58	335	1.91
Total	792	1.54	660	1.28	1087	2.1	890	1.71	1084	2.09	896	1.73	5409	1.74

Table 9.3 Faecal Isolations of verocytotoxin-producing *Escherichia coli* O157 from Humans, United Kingdom 1999 - 2004

Year/	Scotland		England		Northern Ireland		Wales		United Kingdom	
Quarter	Number	Rate#	Number	Rate#	Number	Rate#	Number	Rate#	Number	Rate#
99/1	35	0.69	183	0.37	20	1.19	3	0.10	241	0.41
99/2	97	1.91	224	0.45	3	0.18	15	0.51	339	0.58
99/3	121	2.39	453	0.91	23	1.37	31	1.06	628	1.07
99/4	41	0.81	158	0.32	6	0.36	17	0.58	222	0.38
Total	**294**	**5.80**	**1018**	**2.05**	**52**	**3.10**	**66**	**2.25**	**1430**	**2.45**
00/1	15	0.30	62	0.12	9	0.53	3	0.10	89	0.15
00/2	56	1.11	240	0.48	11	0.65	8	0.27	315	0.54
00/3	87	1.72	390	0.78	13	0.77	30	1.02	520	0.89
00/4	39	0.77	158	0.32	19	1.13	5	0.17	221	0.38
Total	**197**	**3.89**	**850**	**1.70**	**52**	**3.09**	**46**	**1.56**	**1145**	**1.95**
01/1	19	0.38	68	0.14	1	0.06	5	0.17	93	0.16
01/2	60	1.18	151	0.30	13	0.77	10	0.34	234	0.40
01/3	101	1.99	330	0.66	24	1.42	22	0.75	477	0.81
01/4	55	1.09	176	0.35	8	0.47	6	0.20	245	0.42
Total	**235**	**4.64**	**725**	**1.45**	**46**	**2.72**	**43**	**1.46**	**1049**	**1.78**
02/1	16	0.32	54	0.11	0	0.00	1	0.03	71	0.12
02/2	37	0.73	111	0.22	6	0.35	8	0.27	162	0.27
02/3	131	2.59	261	0.52	15	0.88	23	0.78	430	0.73
02/4	45	0.89	133	0.27	6	0.35	3	0.10	187	0.32
Total	**229**	**4.53**	**559**	1.12	**27**	**1.59**	**35**	1.19	**850**	**1.44**
03/1	20	0.40	44	0.09	1	0.06	0	0.00	65	0.11
03/2	31	0.61	128	0.26	4	0.23	4	0.14	167	0.28
03/3	65	1.29	391	0.78	46	2.70	9	0.31	511	0.86
03/4	32	0.63	93	0.19	2	0.12	6	0.20	133	0.22
Total	**148**	**2.93**	**656**	**1.31**	**53**	**3.11**	**19**	**0.64**	**876**	**1.48**
04/1*	15	0.30	48	0.10	3	0.18	3	0.10	69	0.12
04/2*	40	0.79	167	0.33	4	0.23	2	0.07	213	0.36
04/3*	108	2.14	353	0.71	5	0.29	11	0.37	477	0.81
04/4*	46	0.91	112	0.22	7	0.41	5	0.17	170	0.29
Total	**209**	**4.13**	**680**	**1.36**	**19**	**1.12**	**21**	**0.71**	**929**	**1.57**

Rate per 100,000 population
* Provisional data

F:Gastro/New EFIG 2004/VTEC - UK

It is suggested that the likelihood of persons being in contact with cattle faeces relates to the number of cattle and sheep in a region per human population. Cattle and sheep numbers from the Scottish Executive farm census are included in Table 9.4. Because sheep are approximately five times more numerous than cattle, cattle numbers were

multiplied by five, added to the sheep numbers and devided by the human population in each health board area or country to give the livestock to human ratio.

Table 9.4 Proportion of cattle & sheep to humans compared to VTEC O157 notifications per 100,000 population by health board area in Scotland and Scotland compared to England & Wales

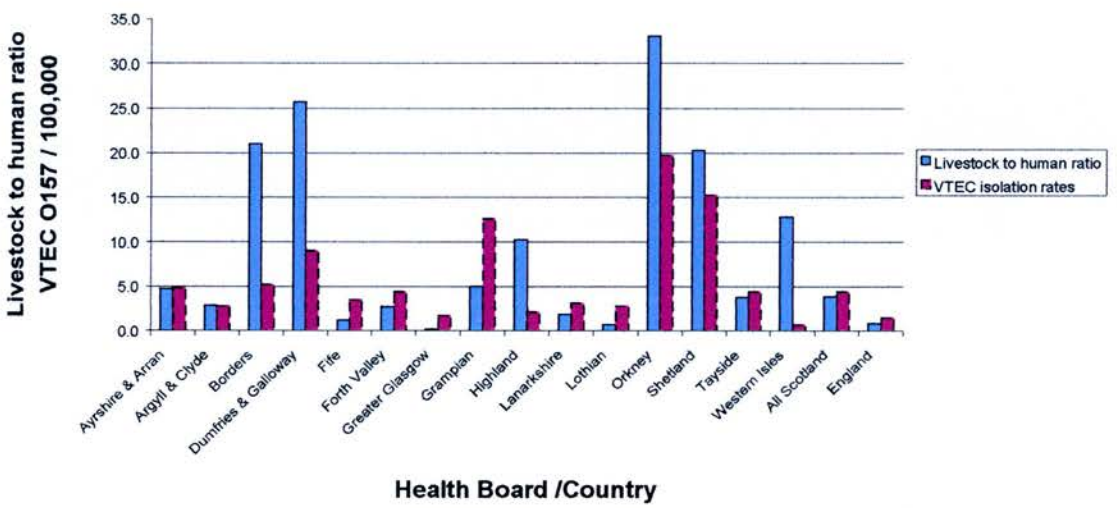
Health board area	Human population	Cattle Numbers	Sheep Numbers	Cattle & sheep / human ratio[1]	VTEC rate /100,000 Population[2]
Ayrshire & Arran	370,833	219,057	655,368	4.7	4.8
Argyll & Clyde	421,428	99,724	695,159	2.8	2.8
Borders	107,692	154,360	1,492,532	21.0	5.2
Dumfries & Galloway	148,889	452,112	1,577,470	25.8	9
Fife	348,571	59,508	122,125	1.2	3.5
Forth Valley	281,818	66,094	427,334	2.7	4.4
Greater Glasgow	858,824	19,844	54,057	0.2	1.7
Grampian	526,984	378,987	758,111	5.0	12.6
Highland	209,524	154,900	1,367,924	10.2	2.1
Lanarkshire	548,387	122,750	421,057	1.9	3.1
Lothian	785,714	55,217	282,081	0.7	2.8
Orkney	19,289	95,523	159,759	33.0	19.7
Shetland	22,222	6,028	420,547	20.3	15.3
Tayside	386,363	129,637	830,423	3.8	4.4
Western Isles	28,571	7,733	328,787	12.9	0.7
All Scotland	5,065,109	2,021,474	9,592,734	3.9	4.4
England	50,000,000	5,612,361	15,704,447	0.87	1.52

Notes

1. Cattle & sheep to human ratio was calculated as total cattle X 5 + total sheep / total humans.
2. 1999-2003 five year average.

The relationship between the ratio of livestock to humans and the rate of reported cases is shown in Figure 9.4 . There appears to be a close relationship except in the Western Isles, where the rate of human VTEC infection is very low and the numbers make the analysis unlikely to be statistically significant. Working on the hypothesis of a direct relationship between the livestock to human ratio and the rate of human infection, the graph predicts almost perfectly the rate in England compared to Scotland. This is further evidence that the majority of VTEC infections are related to humans having contact with cattle or sheep faeces. It is known however that approximately 15% infections are due to human to human spread.

Figure 9.4 Comparison of livestock to human ratios with VTEC O157 isolation rates by health board area in Scotland and between Scotland and England



Eliminating those health board areas with less than 250,000 poulation a graph was drawn to investigate the correlation between cattle population in an area compared to the incidence of VTEC O157 in humans (Figure 9.5). There is an apparent correlation. A second graph was drawn taking into account the possible influence of sheep as well as cattle (Figure 9.6).

Figure 9.5 Comparison of human incidence of VTEC O157 with cattle to human ratio in Scotland by Health Board areas with greater than 250,000 population

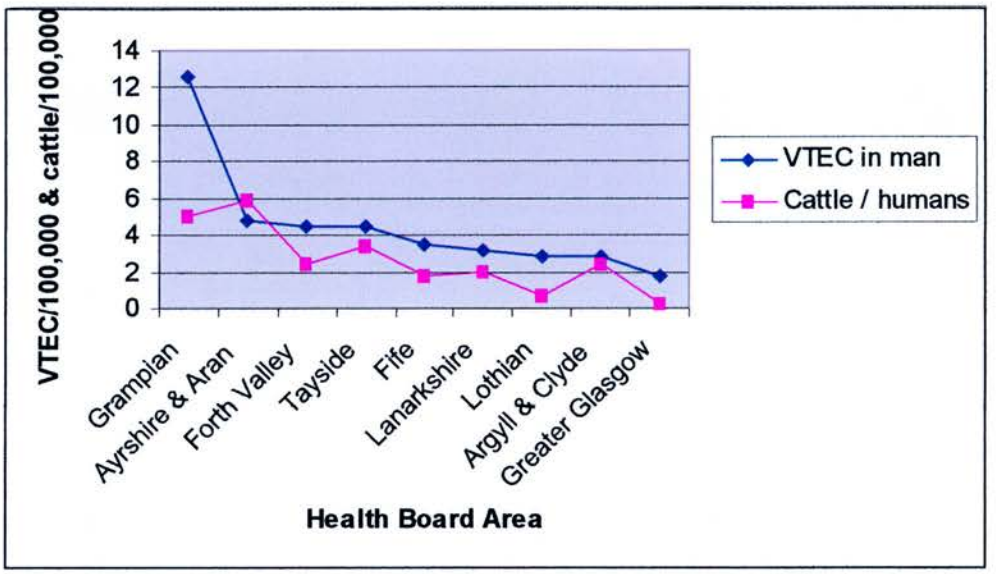
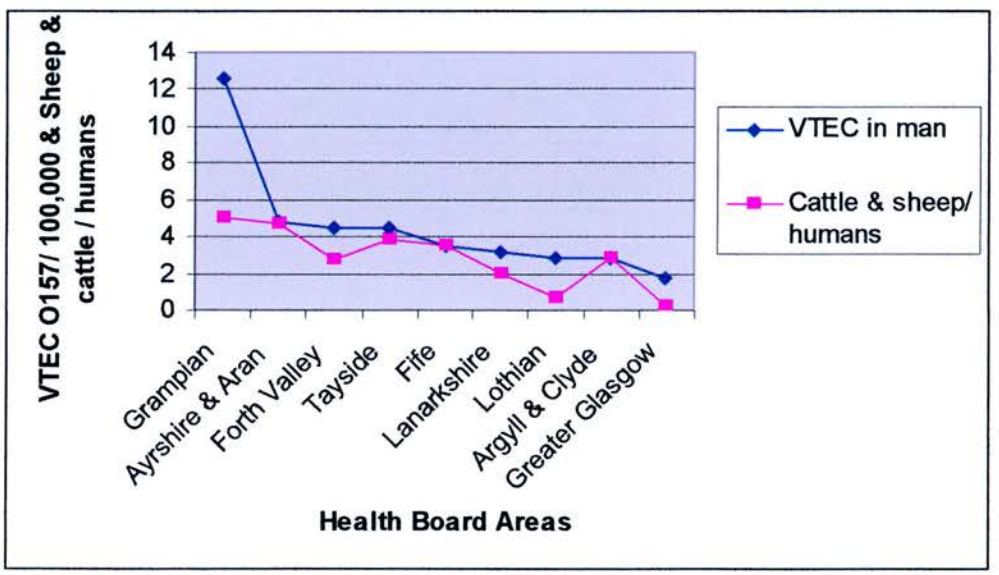


Figure 9.6 Comparison of human incidence of VTEC O157 with cattle & sheep to human ratio in Scotland by Health Board areas with greater than 250,000 population



Statistical analysis

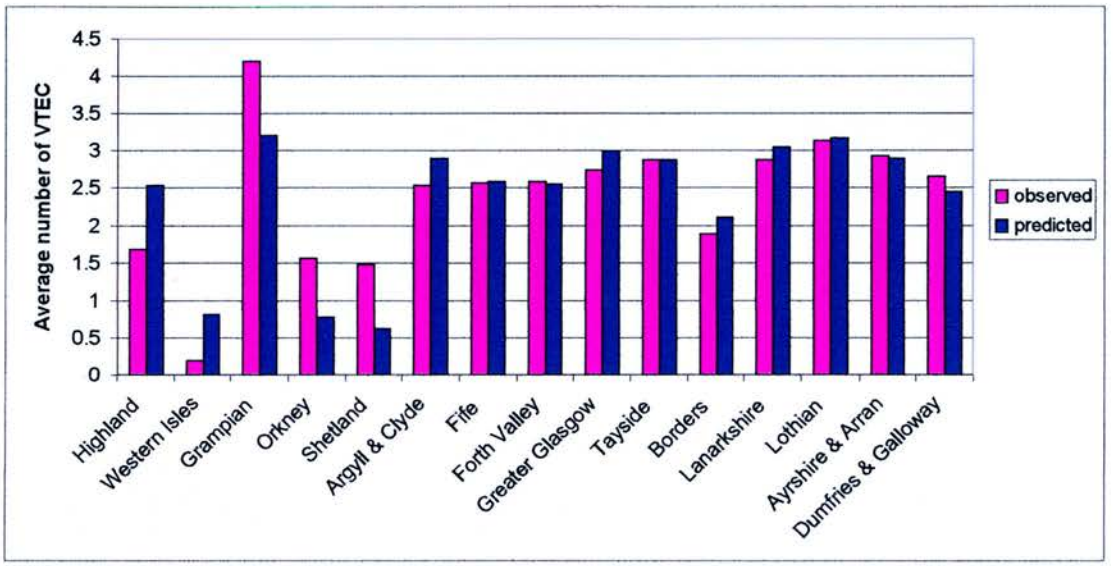
The objective was to develop a predictive equation to explain the difference in VTEC O157 incidece in Scotland and England. The following variables were considered: Human population numbers, cattle numbers, sheep numbers, region, total average and rate of VTEC. The data encompassed 15 health boards within Scotland plus all Scotland and all England.

The first step was to investigate the correlation of all variables. There is a significant correlation between two of the explanatory variables: cattle and sheep. This can lead to problems of multicolinearity. Checking the tolerance and variance inflation factors showed no strong evidence of multicolineaarity, so both cattle and sheep remained in the model. The second step was to run a multiple regression model using SAS version 9.3.1. Animal Health District was included as a random variable to account for any regional differences in averaage number of VTEC infections. The fixed term included: number of humans, cattle and sheep. Data on the average number of VTEC infections was ln(x+1) transformed to correct for heterogeneity in the data. Data on numbers of humans, cattle and sheep was also transformed using ln(x) transformation. The model converged and analysis of the residuals suggested an adequate fit to the model. The final model gave the equation:

Log(vtec_ave+1) = -7.8486+(.6529*loghuman)+(.1153*logcattle)+(.07216*logsheep)

Of the fixed effects, only human was significant ($P<0.001$). The intercept was also highly significant ($P<0.001$) which suggests that there are other factors that are significant but not included in the model. A histogram of the model fit is shown in Figure 9.7. As can be seen the model fits the data in all areas except Highland and Western Isles (where the model overestimates the number of VTEC) and Grampian, Orkney and Shetland (where the model underestimates the number of VTEC).

Figure 9.7 Comparison of the observed and predicted human incidence of VTEC O157 in Scotland by Health Board by multiple regression model



The objective to be able to suggest the differences in rate of VTEC infection between Scotland and and England can not be achieved with this model as the data points are out of range. To do a comparison it will be necessary to collect similar data for England as has been done in Scotland.

Discussion

A stong association between VTEC rates and the ratio of beef cattle to human population has been observed in Canada (Valcour, *et al.*, 2002). The prevalence study (chapter 5) showed approximately one quarter of farms to have at least one shedding animal and the longitudinal study (chapter 4) showed that when farms were repeatedly sampled twelve times 88% farms had at least one shedding animal. As discussed earlier the prevelences detected in these studies by the national standard method are likely to be under estimates of the true prevalence. Since there is close contact with

animal faeces on most farms it is likely that other factors come into play in the establishment of human infection. It is clear that if there is immunity that this should not be relied upon as frequently farmers families have been shown to be infected with organisms from a farm. Strain differences of VTEC O157 isolated from cattle from the studies in this thesis and diseased humans have been reported (McNally, *et al.*, 2001). These workers in University of Edinburgh have suggested differences in the amount of secreted locus of enterocyte effacement (LEE) proteins. Octamer based scanning in the USA has suggested different lineages of VTEC O157 in cattle and man (Kim, *et al.*, 1999). The library of isolates created by the IPRAVE project lends itself well to further studies of this sort.

Sorbitol fermenting VTEC O157 present detection problems, but fortunately are relatively rare. In 2003 the Scottish *E. coli* reference Laboratory isolated three sorbitol fermenting *E. coli* O157 isolates, one of which had VT genes (Locking, *et al.*, 2004, Taylor, *et al.*, 2003). The patient had farm connections and an indistinguishable isolate was obtained by SAC Veterinary Services from a cat. Non-O157 VTEC are now receiving more attention in the UK, being detected in human enteritis (Evans, *et al.*, 2002) and in calves (Jenkins, *et al.*, 2002, Jenkins, *et al.*, 2002, Jenkins, *et al.*, 2003). A prevalence study in Scotland, funded by FSA Scotland, has recently been completed by SAC in parallel with the IPRAVE O157 prevalence study.

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The pathogenicity for calves of *E. coli* with various genes has been reviewed (Mainil, 2000). Only enterotoxigenic *E. coli* (ETEC) are considered to be primary pathogens of calves, while enteropathogenic strains (EPEC), enterohaemorrhagic strains

(EHEC), verocytotoxigenic strains (VTEC) and necrotoxigenic strains (NTEC) are regarded more as opportunistic pathogens.

Other factors such as the numbers of organisms shed could also affect the likelihood of VTEC O157 causing human disease. It is clear from the farm investigations (Chapter 8) that in at least some instances high level shedding contributed to either environmental or water contamination. The concept of some animals shedding high numbers of organisms is likely to increase the number of animals shedding within a group. (Matthews, *et al.*, 2006). The finding of a colonisation site at the recto-anal junction helps to explain how some animals shed very large numbers of organisms (Gally, *et al.*, 2003, Naylor, *et al.*, 2003). As discussed in Chapter 5, the use of rectoanal mucosal swabs as advocated by Rice, *et al.*, 2003, may be a more sensitive method to detect colonised / "supershedder" animals but may not detect transient carriers. An attempt has been made to quantify the reservoir of infection (Strachan, *et al.*, 2005), but for practical purposes all ruminant animals and their faces must be considered a hazard.

While the risk factor analysis for shedding of VTEC O157 by cattle and other animals is of interest, no meaningful attempts have been made to reduce the risks on farm. Concentrating on the prevention of contact with animal faeces, keeping faecal contamination away from playing fields, food and water supplies and educating children to wash their hands are measures more likely to be successful. The successful vaccination of cattle may be superseded by a natural decline in the prevalence of the organism. The possibility of outbreaks and sporadic incidents is likely to continue for many years however. A large foodborne outbreak affecting schools in Wales in 2005

acts as a salutary reminder of the risks to the population. Should there be a flaw in hazard analysis and critical control points (HACCP), i.e. the multiple systems in place to reduce the likelihood of VTEC O157 entering the food chain, there may be potentially disastrous consequences. There is therefore a pressing need to continue to increase our understanding of this organism and the diseases it causes in man and to apply the findings.

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Appendix 1 PUBLICATIONS ARISING FROM THE WORK
(chronological)

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Appendix 2 : RELATIONSHIP BETWEEN THE NUMBER OF ANIMALS IN THE SAMPLING GROUP AND THE NUMBER OF SAMPLES COLLECTED (Chapters 4,5 & 6).

Sample Sizes for 80% success probability

Group Size	Faecal Pat Sampling	Rectal Sampling
10	11	7
15	14	10
20	17	12
25	19	14
30	20	15
40	22	17
50	23	19
60	24	20
70	25	21
80	25	22
90	26	22
100	26	23
200	27	25
300	27	26

Appendix 3 : QUESTIONNAIRE USED IN THE PREVALENCE STUDY (Chapter 5)

CONFIDENTIAL

Farmer's Name:_____ **Date of Visit:**___

Farm Name:_____ **Tel. No.:**_____

Address:_____ **Vet. Practice:**___

_____

Post Code:_____ **CPH No.:**

Farm Type	**Yes / No**
Beef holding with finishing cattle (Suckler beef herds) Dairy holding with finishing cattle Other holding with finishing cattle	

What is the Total Number of Finishing/Store Cattle (age 12-30 months) on Farm at present : _____

How many groups are your finishing cattle kept in ? _____

How many cattle are in the group closest to slaughter/sale ?_____

What is the age range within the group closest to slaughter?_____ ***(youngest - oldest)***

When is this group due to go for slaughter / sale? _____

Where will they be slaughtered / sold? _____

Source of finishing cattle: Do you	**Yes / No**
Breed own Buy in / Purchase	

From what origin are these cattle in this sample group	**Yes / No**
Beef Cow x Beef Bull - Suckler beef Dairy Cow (milking herd)x Beef Bull - Dairy herd Dairy Cow x Dairy Bull - Bull beef	

Questions for Housed Cattle:

Are the Finishing Cattle, the sample group, housed ? **Yes / No**
If sample group are at grass go to 'Questions for cattle at grass' section

	Court / Straw Yard	Slats	Byre	Other
Type of Housing				

When were the sample group Housed? _____

Have the sample group been moved in the last 4 weeks ? _____
Are they in a different pen?

Feeding of sample group	**Yes / No**	**Silage / Other**
Has there been any change in the feeding practice in the last 4 weeks ?		
Is forage being Fed ?		
Type of forage ?		
Are concentrates being fed ?		

Feeding Silage

Is the silage that you feed to the sample group produced on this farm ? **Yes / No**

If Yes please ask following questions	**Yes / No**
Has the silage that you feed this group been produced from fields spread with farm yard manure (animal) ?	
Has the silage that you feed this group been produced from fields spread with slurry (animal) ?	
Has the silage that you feed this group been produced from fields spread with sewage sludge (human) ?	
At any time over the past 12 months have you had a flock of wild geese (large number ~100 birds) on the silage fields ?	
At any time over the past 12 months have you had a flock of gulls (large number ~100 birds) on the silage fields?	

Questions for Cattle At Grass :

When were the sample group Turned out? _____

Have the sample group been moved in the last 4 weeks ?___
Are they in a different field?

Grazing management	Yes / No
Has the land that this group is grazing on been spread with farm yard manure (animal) ? Has the land that this group is grazing on spread with slurry (animal) ? Has the land that this group is grazing on spread with sewage sludge (human) ? At any time over the past 12 months have you had a flock of wild geese (large number ~100 birds) on your pasture ? At any time over the past 12 months have you had a flock of gulls (large number ~100 birds) on your pasture ?	

Do the sample group at grass receive supplement feed **Yes / No**

If Yes please ask following questions	Yes / No	Silage / Other
Has there been any change in the feeding practice in the last 4 weeks ? Is forage being Fed ?		
Type of forage ?		
Are concentrates being fed ?		

Feeding Silage

Is the silage that you feed to the sample group produced on this farm ? **Yes / No**

If Yes please ask following questions	Yes / No
Has the silage that you feed this group been produced from fields spread with farm yard manure (animal) ? Has the silage that you feed this group been produced from fields spread with slurry (animal) ? Has the silage that you feed this group been produced from fields spread with sewage sludge (human) ? At any time over the past 12 months have you had a flock of wild geese (large number ~100 birds) on the silage fields ? At any time over the past 12 months have you had a flock of gulls (large number ~100 birds) on the silage fields?	

Questions for Housed Cattle and Cattle at Grass :

At the time of visit what other livestock is present on the farm ?

Numbers of different livestock on the farm?	Number	Numbers of different livestock on the farm?	Number
Cattle (all other cattle) Sheep (& wintering) Goats Horses		Pigs Poultry Farmed Deer	

What is the source of drinking water for the sample group	Yes / No
Mains Private (Piped water from private burn, spring or well) Natural (burn or river)	

Has the water supply to the sample group been contaminated with faeces in the last 12 months ? **Yes / No**

Is there a possibility that this water supply could be contaminated from	Yes / No
Animals upstream Septic Tank Midden	

Would you agree to another visit if we wished to come back and take a few more samples and ask a few more questions ? **Yes / No**

Would you like to receive your results ? **Yes / No**